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DEPARTAMENTO DE BIOLOGIA ANIMAL

IMPACT OF MINE POLLUTION ON THE ABUNDANCE AND COMMUNITY STRUCTURE OF GROUND-DWELLING SPIDERS (ARANEAE): POTENTIAL USE AS BIOINDICATORS

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MESTRADO EM ECOLOGIA E GESTÃO AMBIENTAL
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Abstract

Heavy metal pollution has been the object of much research in the last few decades, motivated by concerns over human health and the integrity of biological systems. The aim of this study was to assess the impact of mine-originated fugitive dust deposition, as contaminator of surface soils with copper (Cu), iron (Fe) and zinc (Zn), on the abundance and community structure of a Mediterranean ground-dwelling spider community.

Spider and soil bioavailable metal contents were analysed. Lichen data was obtained from models from previous studies at the Cu-mine. Community assessment of ground-spiders was made through pitfall trapping in a series of sites in a 2 km radius area from the contamination source.

Decreased overall community abundance and increased Cu and Fe in spiders were detected with decreasing distance from the mine. Significant correlations between these parameters and high levels of lichens and soil bioavailable metal contents were observed. Cu in spiders did not show a magnitude of enrichment comparable to that of soil; this study therefore agrees with previous studies that Cu seems to be regulated in spiders. Fe in spiders had a even stronger gradient with distance to the mine than Cu, despite the absence of a correspondingly strong soil gradient. Zn soil concentrations in the study area were probably too low for there to be any significant accumulation in spiders.

Ground Hunter guild abundance was found to respond better to soil contamination, while abundance of Specialists responded better to atmospheric deposition. Juveniles, females and males presented different patterns of abundance. The inclusion of juveniles in the overall abundance assessments therefore permits the observation of more generalized and robust patterns for the whole guild and overall community.

The results of the present study indicate that spiders can be adequate bioindicators of soil contamination in the context of a primarily Cu gradient in the Mediterranean ecoregion.

Keywords: heavy metals; Cu; Fe; Zn; spider guilds

Resumo

Os metais pesados ocorrem naturalmente na geosfera. No entanto é com raridade que se encontram na natureza concentrações tão elevadas como aquelas que resultam das explorações antropogénicas de minérios e das suas múltiplas indústrias derivativas.

Nas últimas décadas, a poluição com metais pesados tem despertado a preocupação do público sobre os potenciais efeitos nocivos na saúde humana e na integridade dos ecossistemas, o que tem motivado ao longo dos anos inúmeras linhas de investigação. A utilização de organismos bioindicadores é uma delas.

Dado que uma mesma mistura de contaminantes pode ter efeitos radicalmente distintos em ecossistemas diferentes, é necessário investigar o biota em si para avaliar com algum grau de certeza o impacto e prever o risco que pode advir da poluição com metais pesados.

Os artrópodes têm suscitado grande interesse como sistemas biomonitores, devido à sua abundância, diversidade e capacidade de resposta rápida. De entre eles, a aranhas têm-se destacado pela sua presença mesmo nos locais mais poluídos e pelas grandes quantidades de metais que conseguem acumular sem aparentes danos fisiológicos. No entanto, estes processos de isolamento dos metais das funções vitais (detoxificação) têm custos energéticos que se podem traduzir em reduções de crescimento, fertilidade e taxa reprodutora. As aranhas são portanto boas candidatas a modelos de estudo dos efeito da poluição com metais pesados: são ubíquas (e portanto existe sempre informação, em qualquer tipo de habitat); fáceis de amostrar; e têm um papel essencial no ecossistema (já que são predadoras de topo das comunidades de invertebrados terrestres), podendo reflectir impactos que afectem populações de nível trófico inferior.

Neste estudo pretendeu-se portanto avaliar o impacto da deposição de poeiras com origem numa mina de cobre – no seu papel de contaminante dos solos superficiais da zona circundante com os metais cobre (Cu), ferro (Fe) e zinco (Zn) – na estrutura e abundância de uma comunidade epígea de aranhas da ecoregião Mediterrânica.

Assim, durante o segundo pico de biodiversidade das aranhas em Outubro, foram montadas armadilhas de pitfall numa série de locais distribuídos numa área de 2 km de raio do centro da mina, maioritariamente ao longo dos principais eixos cardinais. Este posicionamento espacial num gradiente de distância com a mina confere uma maior robustez aos dados, particularmente porque, devido aos diversos usos do solo dos terrenos da envolvente da mina (agrícola, pastoreio, urbano) não foi possível uma uniformidade de vegetação nos locais de amostragem.

O uso de pitfalls é conhecido pelo seu enviesamento nas avaliações de abundância, já que favorece os membros da aracnofauna com maior mobilidade, existindo mesmo enviesamento da proporção de machos, fêmeas e juvenis capturados. No entanto, como o propósito da amostragem é a avaliação relativa entre os locais de amostragem, o primeiro enviesamento não é significativo. Quanto ao segundo, dado que poderão existir diferenças de sensibilidades entre estádios e sexo,

foi feita uma análise individualizada a juvenis, machos e fêmeas ao nível da família, da guilda e da comunidade.

Em cada local de amostragem foram também recolhidas aranhas vivas e amostras de solo para análise de conteúdos em metais, representando estes, respectivamente, uma medida da contaminação do biota e da biodisponibilidade de metais nos solos. Para que a influência da deposição atmosférica propriamente dita fosse também considerada, valores para o Índice de Diversidade Liquénica (LDV) e para conteúdos de Cu em líquenes foram obtidos a partir de modelos de estudos anteriores efectuados na mina.

A comunidade epígea de aranhas na zona da Mina Neves-Corvo é caracterizada por uma dominância da família Gnaphosidae (pertencente à guilda das aranhas de Caça Activa no Solo), algo que é característico das zonas Mediterrânicas. A família Zodariidae, da guilda das Especialistas, foi a segunda mais abundante. A estrutura etária e sexual da população capturada em pitfalls no período outonal provou ser dominada pelos machos (53%), seguidos pelos juvenis (35%) e por último as fêmeas (12%). Este enviesamento era esperado, já que os machos e os juvenis são elementos mais móveis que as fêmeas, e logo com maior tendência a serem capturados: os primeiros porque se encontram activamente à procura de fêmeas, e os segundos porque se podem encontrar em fase de dispersão.

Nas análises dos conteúdos de metais, foram utilizadas amostras compostas de espécimes da família Gnaphosidae, as quais permitiram a detecção de relações significativas entre os metais nas aranhas e as restantes variáveis estudadas:

- O Cu nas aranhas apresentou um gradiente de aumento significativo com a proximidade à mina, mas não apresentou uma magnitude de enriquecimento comparável à do solo (as aranhas demonstraram um aumento de apenas 5x, enquanto que o solo viu a sua concentração multiplicar 70x em locais próximos da mina), razão pela qual este estudo corrobora estudos anteriores que sugerem que o Cu seja activamente regulado nas aranhas.
- Um gradiente forte para o Zn nos solos foi observado, no entanto as concentrações mesmo nos locais mais enriquecidos em Zn eram baixas. É pois provável que a quantidade disponível de Zn nos solos da área de estudo seja demasiado baixa para que efeitos nas aranhas ao nível da comunidade fossem detectados.
- O Fe, por outro lado, apresentou um gradiente mais forte com a distância à mina do que o Cu, apesar da ausência de um gradiente de Fe nos solos correspondentemente forte. O aumento dos conteúdos de Fe nas aranhas apresenta mesmo um enriquecimento ligeiramente superior ao do solo, o que poderia sugerir menor regulação; no entanto, porque o Fe tem um gradiente tão forte com a mina, é possível que este esteja a ser activamente incorporado como um mecanismo de alívio da toxidade do Cu: o esclarecimento desta questão mereceria investigação futura.

Relativamente às respostas da abundância, verificou-se que com uma maior proximidade da mina havia efectivamente uma diminuição da abundância de aranhas ao nível da comunidade, e que este era um padrão que se repetia para o aumento dos conteúdos de metais nos líquenes, nos solos e nas aranhas.

As guildas são grupos ecológicos que reúnem famílias que utilizam os mesmos recursos de maneiras semelhantes e podem apresentar, portanto, diferentes padrões de variação. Verificou-se que para a guilda de aranhas de Caça Activa no Solo, a variação na abundância era mais bem explicada pela contaminação do solo; enquanto para as aranhas Especialistas, os padrões de abundância estavam mais relacionadas com a deposição atmosférica em si.

Ao nível das famílias, guildas e comunidade, observaram-se padrões distintos de variação da abundância de juvenis, fêmeas e machos com os parâmetros de contaminação estudados. A inclusão de juvenis nas avaliações da abundância destas comunidades é portanto importante para a observação de padrões mais generalizados e mais robustos para a globalidade das guildas e da comunidade.

Os resultados aqui apresentados mostram que uma análise ao nível da comunidade epígea de aranhas, usando o nível de identificação da família, permitiu a observação de padrões que respondem à contaminação originária da mina. O facto de uma identificação a um nível taxonómico superior surtir resultados é encorajador para um futuro desenvolvimento de um protocolo de monitorização, já que devido aos enormes números de espécimes capturados em pitfall e devido à enorme dificuldade de identificação à espécie e mesmo ao género deste taxon, a obrigatoriedade de identificação a níveis taxonómicos inferiores poderia ser proibitivo em termos de tempo e recursos humanos.

O presente estudo aponta as comunidades epígeas de aranhas como bioindicadores adequados da contaminação do solo no contexto de um gradiente de poluição de Cu na ecoregião Mediterrânica. Mais investigação terá de ser conduzida no futuro na área para verificar os resultados aqui obtidos, ampliando o estudo no tempo e investigando áreas vitais como o metabolismo do Cu e do Fe.

Palavras-chave: metais pesados; Cu; Fe; Zn; guildes de aranhas

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1. Introduction

Heavy metals occur naturally in the geosphere. However, rarely do they reach the considerable high concentrations that result from the human exploitation of metalliferous ores and their industries and other human activities. Although some heavy metals are important micronutrients across all of life – like copper (Cu), iron (Fe) and zinc (Zn) – others are non-essential (Franzle and Markert 2008) and their accumulation in the body is often unhindered by regulation pathways and can more easily become lethal. However, even for those trace elements that are vital to basic physiological processes and regulatory mechanisms exist, excessive assimilation can take place and can lead to reduced fitness and illnesses (e.g. in humans Kabata-Pendias and Mukherjee 2007; Marmiroli and Maestri 2008). Abnormally high contents of these metals in the soil represent therefore alterations to the environment that many organisms may not have the ability to cope, and ecosystem integrity may suffer from it (Kuperman and Carreiro 1997).

Although metal contents in the soil are easy to quantify, the same can not be said for the fraction of these metals that is actually available and transferred from the soil to living systems (Kabata-Pendias 2004; Love and Babu 2006). Due to the diversity and complexity inherent to ecosystems, the same mixture of metal contaminants can have markedly distinct impacts in different systems, making contamination of the biota a hard thing to predict or assess the risk of (Spurgeon et al. 2010). Researchers have therefore long turned to the biological components of ecosystems for direct assessment, and there is a vast literature on the subject, covering many aspects of functional diversity: from nutrient recycling, to decomposition, herbivory and predation (e.g. Butovsky 1996; Hunter et al. 1987a,b,c; Kuperman and Carreiro 1997). Arthropods, with their short life-cycles, rapid responses to environmental changes, great abundances and high diversity of species, physiologies and niches have been natural objects of such studies. Amongst them, spiders have garnered attention, not only due the high levels heavy metal accumulation that have detected for them on the field, but also because they are ubiquitous and exist in great numbers even in heavily polluted sites (Maelfait 1996), making them ideal models for studies on how contamination alters enzymatic biomarkers (Wilczek et al. 2004), life-histories (Hendrickx et al. 2008), growth (Eraly et al. 2010), reproduction (Hendrickx et al. 2003c), behaviour (Eraly et al. 2009), biodiversity and abundance (Bengtsson and Rundgren 1984; Jung et al. 2008; Spurgeon et al. 1996).

The high bioaccumulation in spiders is attributed simultaneously to the way they feed (Maelfait 1996) and the balance between their metals assimilation and excretion rates (Hendrickx et al. 2003a). When then feed, spiders use of digestive fluids to liquefy the soft tissues of their prey, which are then sucked through the mouth opening, and so the hard exoskeletons of arthropod prey are entirely discarded (Foelix 1996). Because heavy metals are usually stored in the soft tissues, spiders ingest higher concentrations of metals that those existing in the whole prey (Maelfait 1996; Tyler et al. 1989).

The midgut diverticulae of spiders is very efficient (Foelix 1996; Hendrickx et al. 2003a; Lee et al. 1978), and high assimilation rates have been detected for the only two elements that were subject of such scrutiny: Fe (Lee et al. 1978) and cadmium (Cd) (Hendrickx et al. 2003a).

Detoxification mechanisms in spiders include: immobilization of metals by development of storage organs, namely intracellular granules in midgut cells (Hendrickx et al. 2003c; Hopkin and Martin 1985); excretion by moulting (Lee et al. 1978) or lyses of midgut cells (Bengtsson and Tranvik 1989; Tyler et al. 1989); and enzymatic change (Eraly et al. 2010; Wilczek et al. 2004). The mechanisms, however imply costs (Marczyk et al. 1993) and, therefore, trade-offs: allocation of energy to detoxification processes translates into less energy that can be allocated to growth and reproduction, resulting in smaller body sizes and fewer eggs (Hendrickx et al. 2003c; Hendrickx et al. 2008). Excretion of metals in spiders was found to be much slower than assimilation for spiders (Wilczek et al. 2004): 6 months being the time necessary for 50% of ingested Fe to be excluded from the body of a mygalomorph spider (Lee et al. 1978) and no excretion at all of Cd being detected in a period of 70 days (Hendrickx et al. 2003a).

Although spiders have very marked seasonal abundance peaks in temperate (Hunter et al. 1987b; Marc et al. 1999) and Mediterranean climates (Cardoso et al. 2007b; Chatzaki et al. 1998; Jiménez-Valverde and Lobo 2006), they are present throughout most of the year, and can inclusively be key prey items for higher trophic levels in the seasons where other arthropods are absent or very poorly represented, which in contaminated habitats can translate into higher quantities of contaminants entering the higher trophic levels seasonally (Hunter et al. 1987b,c). Spiders have therefore the advantage over other arthropod groups, in that they have the potential of being used in seasonal monitoring studies, with important implications for assessment of risk of higher trophic levels.

Additionally, spiders follow all of the criteria defined by Edwards et al. (1996) for the use of key taxonomic groups in the evaluation of soil contamination. Namely, spiders: i) play a key role in ecosystems, as they are top-predators of invertebrate communities, exerting a control over the abundance of insects and other arthropods and reflecting changes in their prey-communities; ii) are present in a wide range of soil systems, as they occupy nearly every terrestrial habitat and are present in all continents but Antarctica; iii) exist in large and dominant populations; iv) are testable under natural conditions; v) have efficient, readily available and non-laborious methods of assessing populations (Foelix 1996; Maelfait 1996; Marc et al. 1999; Spurgeon et al. 1996; Wise 1993).

However, as it was shown for most studied biota in a heavy-metal contamination context (Bengtsson and Tranvik 1989; Janssen and Hogervorst 1993; Straalen et al. 2001; Tyler et al. 1989), spider heavy-metal accumulation is very species-specific (Hendrickx et al. 2004; Rabitsch 1995) and thus subject of much variation, which makes the analysis of contamination patterns difficult on a species to species basis (Hendrickx et al. 2004). A species-level heavy metal assessment of contamination has the additional disadvantage that enough biomass of a particular species, and of a sex within that species – as there are sex-related differences in metal contents (Rabitsch 1995; Wilczek et al. 2004) –, has to be found for all sites: something that is not always possible (e.g. Bengtsson and Rundgren 1984; Rabitsch 1995).

A metal analysis at a higher taxonomic level might average species-specific differences and permit the detection of overall patterns of contamination related metal accumulation. Studies that use order-level or family-level analysis seem to present good evidence of consistent higher accumulation for spiders in more polluted sites (Hunter et al. 1987b; Maelfait 1996), and some present significant correlations with soil metal concentrations (Heikens et al. 2001; Schipper et al. 2008). It is also possible that better correlations with soil may be achieved by restricting studies to the ground-dwelling spider communities, which are more directly exposed to contamination and which probably eat more site-contaminated food then the spiders of higher strata, as the latter feed on higher quantities of flying insects (Rabitsch 1995).

Spider abundance or community structure have been rarely analysed in conjunction with spider heavy metal contents data and soil data; the only ones to our knowledge, were those by Bengtsson and Rundgren (1984) and Hunter et al. (1987b). Studies usually either study metal accumulation by itself (Rabitsch 1995) or species diversity and community composition in relation to soil total contents (Jung et al. 2008; Nahmani and Lavelle 2002), and less often, exchangeable soil contents (Schipper et al. 2008).

Although spider abundance by itself can be misleading in terms of evaluating the impact on a community (as a few resilient species can profit from the decrease in competition and predation from more sensitive ones and grow in numbers), it is an important parameter in assessing the overall contamination of communities and the risks to higher trophic levels, as demonstrated by Hunter et al. (1987a,b,c).

Studies at higher taxonomic levels present unique opportunities in ecological spider studies, as they allow for the inclusion of juveniles in abundance assessments. Juveniles are usually excluded from studies at a species and genus levels, as most can only be safely identified to the family; however, juveniles often constitute the bulk of spider communities and of captures, particularly in certain seasons (Cardoso et al. 2004; Cardoso et al. 2007a; Cardoso et al. 2008a; Jiménez-Valverde and Lobo 2006). While a particular species may only be registered as present during the small window where its representatives reach sexual maturity, it is active and part of the community's composition for longer periods in its juvenile form (Jiménez-Valverde and Lobo 2006). Juvenile can therefore be important in ascertaining the full-breath of the phenology of spider species and seasonal patterns of communities (Jiménez-Valverde and Lobo 2006).

In the context of heavy metal impact assessment, juveniles have sometimes been used in pooled samples where family-level classification was used (Hunter et al. 1987b). However, studies at the species-level have shown differences between relative and total accumulation of metals in juveniles and adults, some of them apparently due to differences in physiology (Maelfait 1996), others clearly due to temporal differences in exposure to metals by ingestion (Hendrickx et al. 2003a). Juveniles could therefore potentially show patterns of response to contamination that are distinct that those of adults due to differences in sensitivity.

Conspecific adults may also exhibit sex-related differences in metal accumulation (Maelfait 1996; Wilczek et al. 2004), but a larger pattern of higher contamination of males, irrespective of species, has also been described (Rabitsch 1995). This higher contamination could be due to the shorter lives of males (Foelix 1996) and to a lower long-term selective pressure. The longer living females, not only may have more time for the slow exclusion of metals, but also be under selective pressures for more efficient methods of regulation of metals, as they are responsible for egg production and the nursing of spiderlings (Wilczek et al. 2004).

Studying the variations in community structure is generally regarded as a good method of measuring anthropogenic impacts, as it reflects alterations of the integrity and functional diversity of biological communities. For spiders, one of the ways of looking at the community structure in a functional way is by the use of guilds (Uetz 1977).

Guilds (*sensu* Root) are defined as groups of organisms that explore the same resource in similar ways; the roles of adaptation to the same class of resources and of interspecific competition in the structuring of communities were central to this original concept of guild (Simberloff and Dayan 1991).

Spiders are exclusive predators and most are generalists; much, therefore, has been discussed and studied of spider ecology on the basis of competitionist theory (review in Wise 1993). This has made spiders obvious candidates for the use of guilds, as they share the same resource (prey arthropods), but obviously have different ways of exploring it (webs, active ground hunting, etc.) (Uetz 1977; Uetz et al. 1999). Guild definition, however, has not followed any one method, and spider guilds have not been consistent from work to work (Uetz et al. 1999), thus casting doubts on the usefulness of the approach in impact assessment. Comparisons, accurate assessments and predictions can only be made if the definition is consensual and if it is valid in its reflection of true ecological units (Simberloff and Dayan 1991).

Until very recently, only the work of Uetz et al. (1999) had used a quantitative method of classifying the different spider families into guilds, providing a framework from which other authors could draw from. Now, an ambitious work by Cardoso et al. (subm.) has used this framework to assign to guilds all known spider families in the world. This new global classification has the potential to solve the problem of multiple guild classifications and can prove to be a valuable tool and framework for ecological spider research. This study will be one of the first works worldwide to use it in the context of environmental impact assessment.

To our knowledge, this will also be the first heavy metal impact assessment study using spiders ever undertaken in a Mediterranean climate, and only the second environmental impact study spider bioindication ever undertaken in Portugal – the first being a study on how different agricultural practices affected spider diversity and abundance in pear orchards (Tavares 2007). The tendency, however, in Portugal and in other Mediterranean countries, is for an increase in such studies, in response to the growing interest and knowledge of spiders as a group and as bioindicators.

Namely, in the last decade, spiders in Portugal were the object of intense surveys led by Cardoso and collaborators. These surveys had the double purpose of characterizing the largely unknown Portuguese arachnofauna (Cardoso and Morano 2010) and gathering data that would allow the development of a standardized semi-quantitative sampling protocol that would allow the evaluation of conservation priorities regarding this much forgotten, but megadiverse taxon, and therefore contribute to the fulfilment of globally assumed comprises to reduce diversity declines worldwide (Cardoso 2008).

The Mediterrean itself is still much an unexplored ecoregion, as the majority of published studies in mine and heavy metal impact assessment have been dedicated to temperate communities with very different characteristics. The active Neves-Corvo copper mine in the Alentejo region presents in this context an excellent opportunity for study. It is an exploration that serves as an excellent case study because, unlike what happens with other explorations (e.g. Filzek et al. 2004; Pinho et al. 2008), it is the sole source of significant pollution in the area, as well as being characterized by a strong contamination gradient that has resulted of decades of activity (Branquinho et al. 1999).

- ***Main goals***

This study aims therefore to assess the impact of mine-originated atmospheric fugitive deposition, in its role as contaminator of surface soils, and of other diffuse mine-related anthropogenic impacts, on the abundance and community structure of a Mediterranean ground-dwelling spider community. Cu, Fe and Zn, metals which result from the exploration of the pyritic ores used in the mine, were chosen as the analysed heavy metals.

Community parameters at a family-level – abundance, guild structure – will be analysed in conjunction with a series of contamination parameters, namely: heavy metal contents in spiders at a family-level; soil bioavailability of metals; and heavy metal atmospheric deposition as measured by lichens. Juvenile, adult female and male abundances will also be analysed separately and together, so differences in patterns could be detected.

- ***Framing study in Master Degree***

Environmental impact assessments and monitoring studies are essential tools for the sustainable management of natural resources and human activities. The growing awareness and recognition of the importance of the ecological component of these evaluations has led, in recent years, to increasing research on biological indicators and to the generalization of their use.

Spiders have the potential to be large spectrum bioindicators, since not only do they reflect the variations that occur in the communities of terrestrial arthropods on which they prey, but they are also ubiquitous, rapid colonizers and respond positively to the growing complexity of their natural habitats. Sampling spiders is also cost-effective and large samples have little impact on their populations, so monitoring protocols can feasibly be established in the future.

2. Materials and Methods

2.1. Study area

The study was conducted in the underground copper mine at Neves-Corvo, Castro Verde, in the southern region of Alentejo, Portugal [Figure 1]. Neves-Corvo is the only copper mine regularly in operation in Portugal and among one of the most productive copper explorations in the world.

The mine produces Cu-concentrate and Zn-concentrate mainly through sulphide ore processing. The main sources of atmospheric pollution in the area are the open-air concentrate stockpiles of Cu and Zn, as well as the waste heaps. The Cu-stockpile is composed mainly of chalcopyrite ($CuFeS_2$) with high Cu (24%), Fe (31%) and S (35%) levels. The waste heaps, which have Fe, have no significant amounts of Cu (Branquinho et al. 1999).

Figure 1. Photo of the Neves-Corvo mine and surrounding landscape. The waste heaps (grey mounds) are clearly visible from afar. This photo was taken in Spring 2009, 800 meters south of the centre of the mine.

The two stockpiles, the waste heaps and the dust caused by traffic around the mine are the main sources of particulate atmospheric pollution, and, specifically, of heavy metal pollution. This region is warm, with a semi-arid climate (characterized by an annual total rainfall of 400–500 mm and air temperatures ranging between 4 and 32 ºC). The dominant winds blow from NW-W to SE-E [Figure 2]. The total heavy metal contamination of the soils due to this particle deposition is hard to ascertain by soil sample analysis alone as soils may reflect the chaotic distribution of pyritic ore that is characteristic of the area rather than the mine-originated contamination. For this reason, studies with lichens as bioindicadores of atmospheric pollution have been used in the impact assessment of the mine on the surrounding landscape since as early as 1994 (Branquinho et al. 1999). The existence of these studies was instrumental in the choice of location, as previous data of a well-established bioindicator as are lichens, which can count with more than half a century's utilization worldwide (Conti and Cecchetti 2001), could provide us with a comparative framework for the spider data.

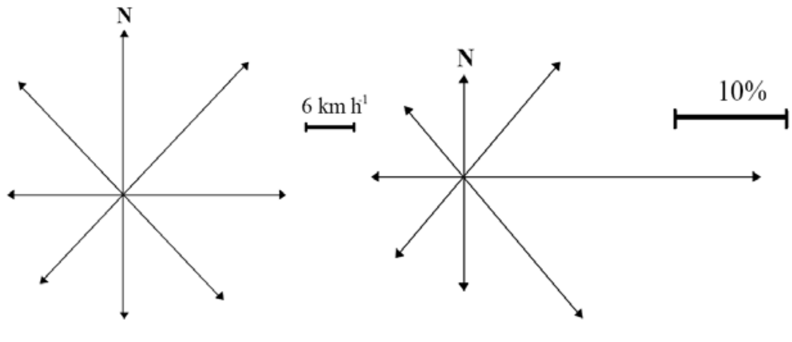

Figure 2. Wind speed (left) and wind frequency (right) referred to 1951-1980 at the nearest meteorological station Beja (approx. 45 km NE from the mine). Taken from (Branquinho et al. 1999).

2.2. Land use and vegetation

The mine is located in a rural area, far from any other industry or any large villages, and there are no other significant sources of pollution at a local level. The landscape is dominated by low density *Quercus ilex* L. woodlands. Understory is sparse and is only continuous within the occasional rockrose (*Cistus ladanifer* L.) dominated patches. The litter layer is mostly absent. Land uses within the study include the industrial (associated with the mine), the urban (small villages), the agricultural, the grazing of cattle, as well as small game hunting.

Spiders have been extensively described in the literature as responding both in terms of diversity and abundance to habitat structural complexity (namely abundance of ground shelters, litter depth, arboreal cover and understory continuity – e.g. Cardoso et al. 2007b; Rypstra et al. 1999; Uetz 1977) and land use and agricultural practices (Rypstra et al. 1999). The mechanisms by which this response operates are still unknown, so prediction of general responses to one or another habitat structure feature or the type of feature that may represent the most important in a given setting are matters of speculation (Rypstra et al. 1999).

However, the reality is that in heavy metal impact assessment studies, uniformity of vegetation may not be achievable, as industrial zones that are sources of contamination can be intersected by other land uses (e.g. Filzek et al. 2004) and because alterations of vegetation structure and species composition can be a result of contamination and count as secondary source of impact (Eeva et al. 2004; Hunter et al. 1987a; Maelfait 1996).

In the one case where two different vegetation types were sampled along a strong contamination gradient, the data from the two types was pooled for each site, as no significant differences could be found between them, i.e. the contamination dictated overall patterns and overrode the effect of vegetation (Bengtsson and Rundgren 1984).

A qualitative characterization of the sites regarding land use and habitat structure was made nonetheless, so as to account for possible cofounding variables. Because a more quantitative characterization (based on the measurement of microhabitat variables and on the quantitative and qualitative description of the vegetation) would go beyond the scope of this work, a qualitative habitat characterization of all sites was made instead. This characterization includes both land use categories (tillage or no tillage, trampling, general land use) and habitat structural complexity categories. In the latter, to the more usual categories of litter, herbaceous, shrub and arboreal layers was added a comparative assessment of the abundance of ground shelters. To these five categories a score was given based on the comparison of all sites to two sites considered to have the highest and the lowest score for a given category. The sum of these five scores constituted a Habitat Structural Complexity Score (HSCS) [see Annex I, Table 2].

The spatial arrangement of our sampling points, as already mentioned, gives a certain robustness to our data, in that a spatial pattern of contamination exists (Branquinho et al. 1999), but a pattern of habitat complexity and land use does not seem to [see Annex I, Table 1].

2.3. Ground-spiders sampling

Ground-spider sampling took place in two distinct periods of 2009: from the end of May to beginning of June (Spring); and from the beginning to the end of October (Autumn). These periods correspond to the two peaks of spider biodiversity identified for Portugal by Cardoso et al. 2007b. Sampling in distinct seasons had also the purpose of covering some of the probable seasonal variation of the impacts of the mine on the terrestrial arthropod communities.

The Spring sampling period was characterized by a mean temperature of 18.45±9.1ºC (max.: 36.0; min: 7.5) and a precipitation of 0.5 mm (max.: 7.4; min: 0.0), whereas the Autumn sampling period was characterized by a mean temperature of 19.93±7.5ºC (max.: 32.0; min: 9.0) and a precipitation of 2.7±10.2 mm (max.: 54.2; min: 0.0) [Data from the mine's meteorological station].

An average of 15 sites was used for each seasonal period, distributed in a 2 km radius in every direction from the Cu-concentrate pile. The 2 km delimitation was based on information from the lichen studies at Neves-Corvo, in which these bioindicators showed that the influence of the mine significantly decreased at an average distance of 1,5-2 km. From that distance outwards, all variation in the Air Quality Indicators for lichens were considered to be normal (Branquinho et al. 1999; Branquinho and Pinho 2005).

The spatial placement of the sampling sites sought to be: 1) as close as possible to previous lichen sampling points, for a better data comparison and correlation; 2) denser nearer the copper exploration, where higher variation and impact were expected; 3) distributed mainly along the four directional axis, with some additional points in intermediate spatial positions, so as to cover as much of the area as possible; 4) as far as possible from any roads and human habitations, i.e. local sources of disturbance; 5) in fields with intermediate to low year-round human activity.

However, the mixed uses of the land did not allow for all of the sites sampled in Spring to be used again in Autumn [Figure 3]. Nor did the spatial nature of our sampling, in conjunction with our other requisites, permit the choice of similar patches of vegetation in which to set the pitfall traps, as has been done in similar studies (e.g. Bengtsson and Rundgren 1984; Hendrickx et al. 2004; Hunter et al. 1987b).

Pitfall trapping was chosen as the ground-dwelling spiders capture method, as it is the one of the two methods directed at epigeous spiders described by Cardoso (2004) that requires the less experience, effort and cost to employ (Cardoso et al. 2007b): all desirable aspects in any impact assessment protocol. Additionally, it is a method that can capture almost half the spider species living in a typical Mediterranean habitat (Cardoso et al. 2007a) and that can capture a great number of individuals (Cardoso et al. 2008a), which is important for the statistical robustness of the data. This method, however, is known for its bias where abundance and community structure characterization are concerned, as captures are dictated by the joint effects of density and trappability (Melbourne 1999; Spurgeon et al. 1996). For this reason some authors have described its measurement of abundance as 'activity abundance' or 'density abundance' (Melbourne 1999; Uetz 1977); for simplicity, however, we will refer to the number of individuals captured in pitfalls only as 'abundance'.

For spiders, the use of pitfalls translates into two main biases: 1) sexually mature males in active search for a mate, and juveniles in their dispersion phase are more likely to fall in pitfalls than the generally less mobile mature females; 2) species that are more active on the ground and that have larger body sizes are more likely to be caught and therefore more likely to be overrepresented in the overall community structure (Lang 2000; Topping and Sunderland 1992). As for the first bias, the separate assessment of juveniles, adult females and males will permit us to identify situations where this bias might have an influence. Concerning the second bias, as the purpose of this study is the relative comparison between sites and not its complete characterization, the first bias will likely have little impact on our results, especially at such a local scale: within an area of 4 km of radius, since the overall vegetation and land uses are the constant, we can assume we are talking of the same spider community. Spiders have been extensively shown to be apt colonizers over distances of hundreds of kilometres via ballooning (Foelix 1996; Greenstone et al. 1987), and indeed this has been found to be the principal colonization process in agrosystems (Bishop and Riechert 1990), where tillage and changing crops lead to cyclical reduction of spider communities and propitiate re-colonization phenomena. However, bordering habitats can also contribute as sources of colonizing spiders if they are physiognomically similar to the ploughed fields. For less vagile families, the rotative nature of pasture producing tillage in Neves-Corvo allows for temporary refuges to exist at all times. So, despite the probable slight differences in species composition at the level of the microhabitat, overall species composition in the study area would be expected to be same if not for the presence of the Neves-Corvo mine. If a pattern can be detected spatially in relation to the mine, this means that there is an impact on the spider-community that supersedes micro-habitat variation.

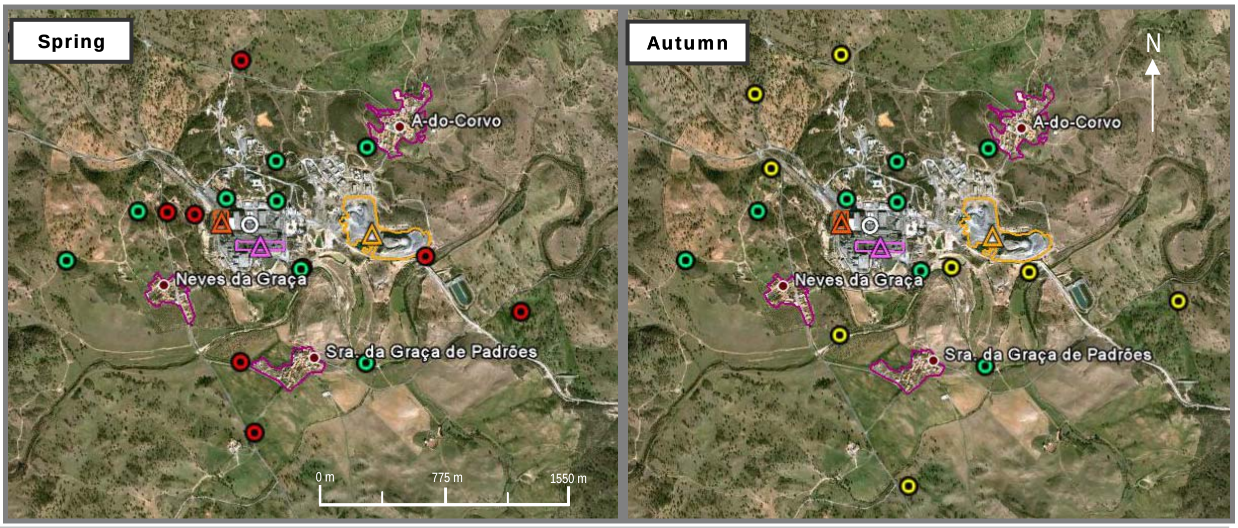

Figure 3. GoogleEarth© photo of the study area. Sampling sites are represented by coloured dots (⦿): red for sites used only in Spring (n=7), yellow for those used only in Autumn (n=8), green for those used in both seasons (n=8). The centre of the mine is represented by a white circle (○). Stockpiles are identified by triangles (△) and delimited by coloured lines: red – Cu-stockpile; pink – Zn-stockpile; orange – waste heaps. Villages are labelled with their respective names and delimited by purple lines.

At each site, three rows of 4 pitfalls traps distancing 5 m from each other were set. Each row was 10 m apart from the next, so pitfall traps occupied an area of 300 m^2 [Figure 4a]. Traps stayed on the field for 13 days before being collected and were set twice during each seasonal period, so that each point could count with 24 pitfall samples per season and 48 pitfalls total.

The pitfall traps were made according to the protocol established by Cardoso (2004): two 33cL plastic cups 8 cm wide at the opening were used, one inside the other; the top one was one-half full with car-coolant liquid (preserving liquid) and a few drops of detergent, and the bottom one was empty (for easier replacement of traps); the cups were covered with white plastic plates, supported by sticks, 3cm high from the ground level [Figure 4c].

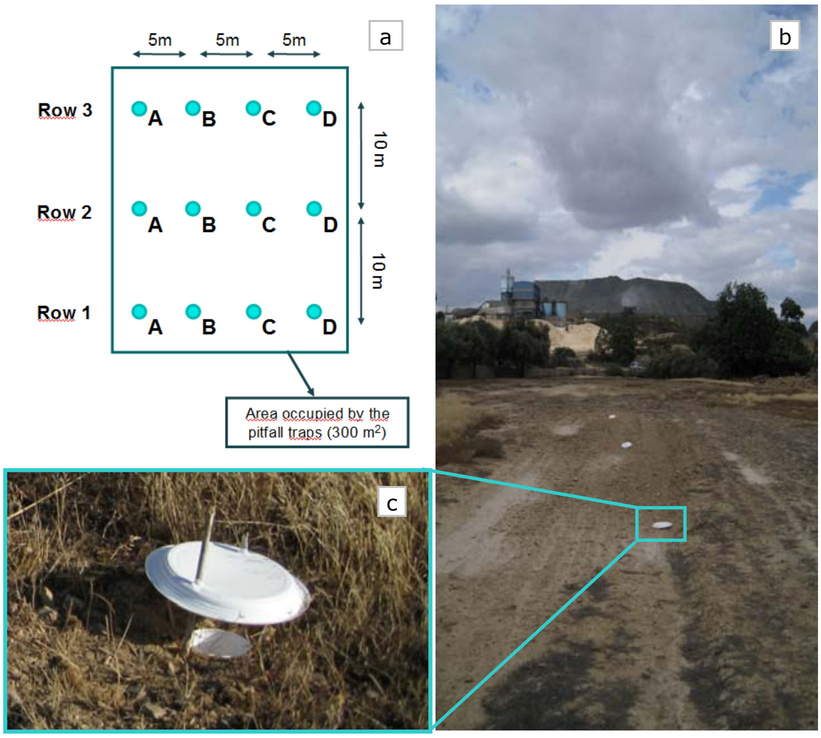

Figure 4. Pitfall sampling in the Neves-Corvo study area: a) pitfall sampling design per site; b) photo of row of pitfalls in sampling site B-04; c) photo of a set pitfall trap in the field (plate was hiked higher on one side so the cup could be clearly seen in the photo).

Due to extreme rainy weather during the first week of October, more than half the traps were assumed to have stopped sampling a few days after being set (as water in the bottom cup caused them to rise above ground-level) or to have lost material by flooding. Hence, only the samples corresponding to second autumn were considered in the subsequent statistical analyses (i.e. 12 pitfall samples per site).

The collected pitfall material was however, even discarding the first sampling of October, too numerous to be processed in conjunction with all the other analysis that needed to be done for the realisation of the studys' goals. And as the Autumn sampling was the only one to have soil and spider heavy metal analysis, it was preferred to the Spring sampling, despite the fact that Spring is the season of higher spider abundance and diversity (Cardoso et al. 2007b). For purely descriptive purposes, 60 pitfalls (corresponding to the 5 sites in a straight line going west away from the mine) out of the 180 pitfalls (15 sites) of the second Spring sampling were processed.

All spiders were discriminated as adult females, adult males and juveniles and identified to family level using the illustrated key of Iberian genera by Barrientos et al. (unpublished document). In the identification of juveniles, only Linyphiidae and Theridiidae presented a problem, as they share a similar physiognomy. Identification without the benefit of genitalia in these families has to be made through the not very easy recognition of diagnosing characters such as the theridiid tarsal "claws" (modified hairs) and labium shape. As linyphiids and some theridiid species are of very reduced dimensions, even as adults, the individuals where identification was dubious were not included in the data. As Linyphiidae is the family that is most often caught in pitfall, juvenile abundance is probably underestimated.

The identified spider families were then assigned to guilds according to Cardoso et al. (subm.) [see Annex I, Table 1]. Guild definition was based on the method used by Uetz et al. (1999), but extended to all spider families in the world. This classification has therefore the potential, not only to be used extensively around the globe, but most importantly to set a standard that will reduced the problems of multiple guild classifications illustrated by Simberloff and Dayan (1991).

As specimens were only identified to family, families for which guild discrimination was made at a sub-family level were assigned to the guild corresponding to the sub-family with greater ground affinity. This was decided on the assumption that, as the method of capture is pitfall trapping, it was far more likely that these individuals belonged to more ground-wandering taxon. Such was the case of families Linyphiidae and Dictynidae: the former was assigned to the Other Hunters guild and the former to the Ground Hunters guild.

2.4. Spider heavy metal analysis

For each of the sixteen Autumn sites, spiders were captured during the first week of November for heavy metal content analysis. Because killing and preservative solutions may influence the dry weight of the specimens and hence alter the metal concentrations found in the body (Hendrickx et al. 2003b), these spiders were captured live by hand and by dry-pitfall trapping. The specimens were then kept in separate containers to avoid cannibalism and then killed and stored in a freezer until heavy metal analysis.

The frozen spiders were dried for 48 h at 60 ºC and weighed to the 0.1 mg. Only the family Gnaphosidae was captured in all sixteen sites and hence, that was the family chosen for the metal analysis.

Most specimens were too small to obtain accurate individual metal and weight measurements (minimum individual dry weight: 0.2 mg; maximum: 12.3 mg). Therefore all Gnaphosidae individuals captured per point, regardless of number or gender, were gathered in a pooled sample, resulting in sixteen Gnaphosidae pooled samples (min.: 1.2 mg; max.: 59.6 mg) [see Annex I, Table 2 for more details].

The pooled samples with a dry weight of 20.0 mg or less were digested in 0.5 mL of 65% HNO_3 solution at 90 ºC; samples with higher dry weights were digested using 1.0 mL of 65% HNO_3 solution, also at 90 ºC. After digestion, distilled water was added to the solution: 1.0 mL and 2.0 mL, respectively.

Metal concentrations were determined by means of flame atomic absorption spectrometry (AAS) (SpectrAA-50, Varian) for Cu, Zn and Fe. All metal concentrations were expressed as µg metal/g dry weight. Quality control was carried out by analyzing the reference materials humus (H467, H468 and H469) and bovine liver (NIST 1577G). Values were within acceptable values and in the same order of magnitude.

2.5. Soil heavy metal analysis

Soil analysis in Neves-Corvo were done by the mine's Environmental Department once before, but the high total content values and the extremely chaotic distribution of the metals of the naturally rich region rendered the analysis useless for effects of impact assessment. Therefore, for a soil measure that could potentially reflect the mine-related activity, as well as providing more biological pertinent information (Kabata-Pendias 2004), we chose to perform a bioavailability analysis.

Soil samples were collected for each of the sixteen Autumn sites at the end of October (on the same day as the last Autumn pitfall samples were collected). After discarding the organic litter layer and the topsoil vegetation layer, the soil surface (depth 3-5 cm) was collected using a small plastic container. Each sample was composed of three sub-samples taken from different sections of the 30m x 20m area delimited by the pitfalls.

Bioavailable heavy metals (Cu, Zn, Fe) determination were done according to Branquinho et al. (2007), except for the EDTA concentration used. Soil samples were cleaned of remaining rock and plant detritus and sieved using a 2mm sieve. Samples were then dried at 60 ºC for 24h and placed in a container with silica to extract the remaining water residues. 2g of the completely dried soil were eluted in 40mL of 5Mm EDTA and the resulting solution agitated at 175 rpm for 3 hours to extract the available soil ions. After a 24h rest period, the samples' Cu, Zn and Fe concentrations were determined by flame atomic absorption spectrometry (AAS) (SpectrAA-50, Varian).

2.6. Lichen data

Environmental impact assessment studies of the Neves-Corvo mine by Branquinho and Pinho (2006; 2005) produced interpolation models of Lichen Diversity Values (LDV), and lichen Cu content (Bioavailable and Particulate) spanning an area of 4 km radius from the mine. While lichen contents will give a measure of atmospheric deposition of heavy metals, LDV will present an integrated index of habitat quality.

Values for all spider sites, which were not exactly the same as the sampling sites used in these studies for the lichens, were calculated using the referred models.

2.7. Distance to the mine

Distances to the Cu-concentrate pile, Zn-concentrate pile and waste heaps were calculated using GoogleEarth (photographs dated from 31 December 2004). However, stockpiles and waste heaps were too close to each other for these distances to be useful in differencing each of the three pollution sources. The distance to the centre of the mine was therefore used in all analysis as a substitute variable unifying all anthropogenic impacts related to the mining process at Neves-Corvo.

2.8. Data treatment and statistical analysis

For each point, as not all 12 pitfalls per site were recovered, the average number of individuals per pitfall was used, rather than the more biased raw counts. Abundance values were therefore calculated as the average number of juveniles, adult females, adult males and total individuals pertaining to each family and each guild captured per pitfall trap.

The proportion of juveniles, females and males was calculated for each family, guild and for the overall spider community. This parameter was used simultaneously as an indicator of the overall life-cycle phase of the families and as measure of impact of contamination on the age and sex community structure within a family or guild.

The proportion of each family within their respective guild per site and the proportions of each family and of each guild to the total number of specimens captured per site were also calculated. All three parameters represent different aspects of the spider community's functional structure.

A series of exploratory analysis of the data followed, using both the graph capabilities of Microsoft Office 2003: Excel and the PCA and the non-parametric correlation modules in STATISTICA 9, to determine the best parameters to use.

Principal Components Analysis (PCA) were made using abundance and community structure parameters were used as variables in the analysis, while contamination variables (metals in soil, lichens and spiders), distance and the vegetation structure index were used as supplementary variables. Land use and other habitat characterization categories were used as grouping variables, so that patterns shown by the PCA that reflected patterns in habitat type could be detected.

Lichen and contamination variables were fitted to bivariate models that had distance independent variable distance. R-square values were examined for statistical significance of all fittings at a confidence level of 95% (N=16; r=0.426; R^2=0.181). Variables were expected to be correlated to themselves, so Spearman correlations were made to ascertain their significance and magnitude.

Abundance and community structure variables were fitted to bivariate models that had for independent variable one of the contamination variables or distance. Only groups with 30 specimens or more in the total Autumn catch were considered. R-square values were examined for statistical significance at a confidence level of 95%. Lichen data (Particulate and Bioavailable Cu and LDV) was best fitted by linear models, while other contamination variables (metals in soil and in spiders) were best served by logarithmic models. As for distance to the mine, linear and logarithmic models had similar results, with only slight deviations. As lichen variables have a logarithmic relation with distance (Branquinho et al. 1999) and were found to have a linear one with community variables, it would be expected for abundance-distance relations to be logarithmic. The logarithmic model was therefore chosen to be used with distance.

Selected community and contamination parameters were then used to rank the 16 Autumn sites. The chosen variables were: overall juvenile abundance; overall female abundance; overall male abundance; soil bioavailable Cu, Fe and Zn; Cu, Fe and Zn in Gnaphosidae. For each variable, sites would be placed in one of four quartiles and given a score accordingly (25 for the worst quality quartile; 100 for the best quality quartile). Triplets of related variables (e.g. Cu, Fe and Zn in Gnaphosidae) would be then summed to give a score (min.: 75; max.: 300) classifying the sites according to that particular set of variables. Triplet scores were then summed into an integrated score of site quality according to soil and ground-spider parameters, with a minimum value of 225 and a maximum of 900. Triplet scores and the integrated score were then compared for similarities in classification of sites.

3. Results and Discussion

3.1. Ground-dwelling community characterization

Despite the great advancements that recent studies present, knowledge of species composition, structure and seasonal abundance variation of spider communities in the Mediterrean region, and specifically in Portugal, must remain a priority, as there is much to be known (Cardoso 2008). For this reason, we wish to add a family-level and age-sex structure characterization of the epigeous spider community of Neves-Corvo to the current knowledge.

A total of 3325 spiders, 1565 of which where captured in Spring (5 sites; 60 pitfalls) and 1760 in Autumn (16 sites; 182 pitfalls), were identified to family-level. Twenty-five families, representing 7 guilds, were found in the Autumn pitfalls and 26 families, in 8 guilds, in the Spring pitfalls, adding to a total of 30 families. Families only found in the Autumn captures were Ctenizidae, Nemesiidae, Palpimanidae and Prodidomidae; while Araneidae, Atypidae, Oxyopidade, Sparassidae and Zoridae were only captured in Spring.

The sex ratio in Spring was 1:1 (N_{males}=246; $N_{females}$=212) and in Autumn 4:1 (N_{males}=930; $N_{females}$=213). The reason for the equality in the Spring ratio, when a higher male capture rate in pitfall was to be expected, and was indeed obtained in Autumn, can be explained by the fact that this is a community overall sex ratio. Species which have passed their reproductive period, and for which most males have died (as most males have a short life after sexual maturity: no more than three to four weeks. Foelix 1996), might accuse more females, either by comparison or because they are active in post-copulatory dispersal. In the same way, species which have just started their reproductive period will have a large proportion of males, which sometimes mature first in a strategy to find females just before their last molt, and therefore still virgins, and drive away other suitors while they wait for them to mature (Foelix 1996).

The proportion of juveniles in total spider captures is not always mentioned in studies at a species-level where juveniles were discarded, nor in studies at family-level where they were used. However, in studies were such a mention is made, the proportion of juveniles varies widely: from 5% in pitfall traps and 67% in density sampling in a May to October study in a wheat field in the UK (Topping and Sunderland 1992); to 92% in a sweep, beat and pitfall sampling in a year-round study targeting Araneidae and Thomisidae in Spain (Jiménez-Valverde and Lobo 2006); to 75% in a ground, sweep and pitfall May catch in a scrubland in southern Portugal (Cardoso et al. 2007a); to 69% in a May to July multi-method sampling in a cork-oak woodland in central Portugal (Cardoso et al. 2008a); to 28% in pitfall traps and 53% in the other five sampling methods used in a early June study in a oak woodland in northern Portugal (Cardoso et al. 2008b).

In the case of the Neves-Corvo sampling, the juvenile proportion was 70% (N=1107) in Spring and 35% (N=617) in Autumn [Figure 5]. If Topping and Sunderland's (1992) conclusion that pitfall traps capture a disproportionately higher amount of adults holds true for southern Portugal in both these seasons, the actual juvenile abundances would be expected to be much higher than those presented here. However, the Spring juvenile proportion is in accordance to the data presented by Cardoso et al. (2007a), in which two other methods were used, and in accordance with the author's conclusion that populations in structurally simple habitats (as are those of Neves-Corvo) present narrower peaks of adult abundance, resulting in higher percentages of juveniles in samples.

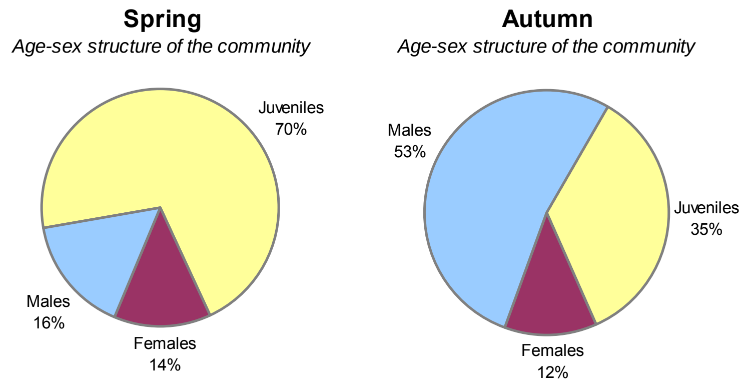

Figure 5. Age-sex structure of the ground-dwelling community as captured by pitfall in Spring and Autumn.

The lower juvenile proportion in Autumn is harder to explain for lack of isolated seasonal data to compare it to; however, after the diversity peak in May to June, it is expected that most species would have successfully produced offspring. These juveniles will then have to face the harsher season in a Mediterranean climate: the dry summer (Jiménez-Valverde and Lobo 2006). A high mortality may therefore be expected, especially since we are talking about an arthropod group (Norris 1999). This in conjunction with the sexual maturation of Autumn-reproducing species (Cardoso et al. 2007b) and the lower prey-abundance typical of this period, may explain the lower juvenile proportion detected for this period.

This does suggest that juvenile abundance does not accompany conspecific adult abundance variation, as noted by Norris (1999). Norris also points out the possible problem of weighting juveniles the same as adults in abundance evaluations groups where high mortalities of juveniles and high inter-annual variations are expected: the inclusion of juveniles may obscure patterns or suggest misleading ones in impact assessment studies. His recommendation was to either exclude them or treat them separately. Although we agree that treating juveniles separately is a sound recommendation until more is known about spider communities, we disagree that they should be discarded, particularly in impact assessment studies, as juveniles are an integral part of the spider community and of the food web, as well as possibly presenting different sensibilities to contamination than adults.

If juvenile abundance, in the context of an impact assessment study, is comparatively low at a site and if this is a trend maintained through time, then this information is relevant as it may be evidence of a negative response, either in terms of higher juvenile mortality or of lower reproductive output. Juveniles can also add to the understanding of the phenology and seasonal variations in the communities, as they represent a continuum state that leads to adulthood (Jiménez-Valverde and Lobo 2006): and these variations are the object of impact assessment studies.

In this study, different sex ratios, and indeed slightly different juvenile-to-adult ratios, were found for different families, suggesting that their representative species might be undergoing different phases of their life cycle. However, the predominant trend in Spring is for juveniles to be the most abundant component of the age-sex structure of the families, while in Autumn males are the most abundant [Figure 5]. Species that reproduce exclusively in autumn (stenochronous of autumn) might overwinter as eggs or as freshly hatched juveniles, therefore contributing to the spring juveniles (Marc et al. 1999).

Gnaphosidae and Zodariidae were curious cases as they provided in both seasons a quite a few representatives of juveniles, females and males [Figure 6], unlike other families in which one of the three components was often missing. If one compares family relative abundance in Spring and Autumn using the three common sites in both samplings [Figure 7], we can see that they are two of the most abundant families in the community, which might make them good targets for seasonal age and gender oriented impact studies in the future.

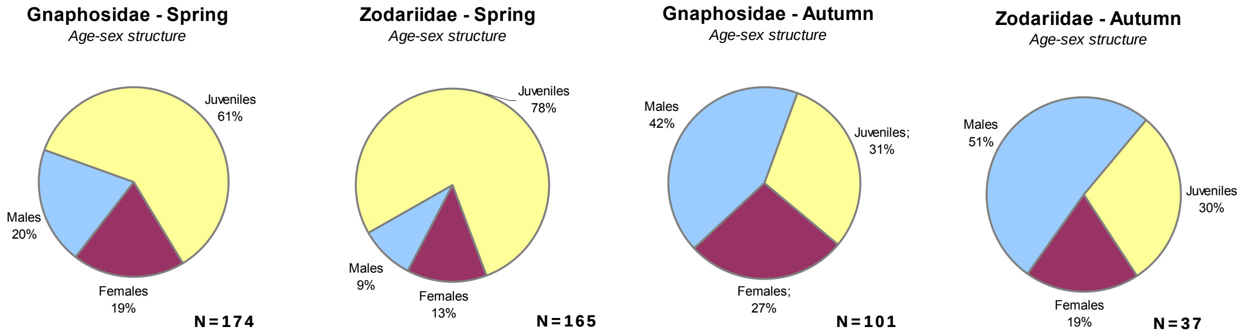

Figure 6. Seasonal shifts in the age and sex structure of Gnaphosidae and Zodariidae. For valid comparison, only the 3 common sites for Spring and Autumn were used.

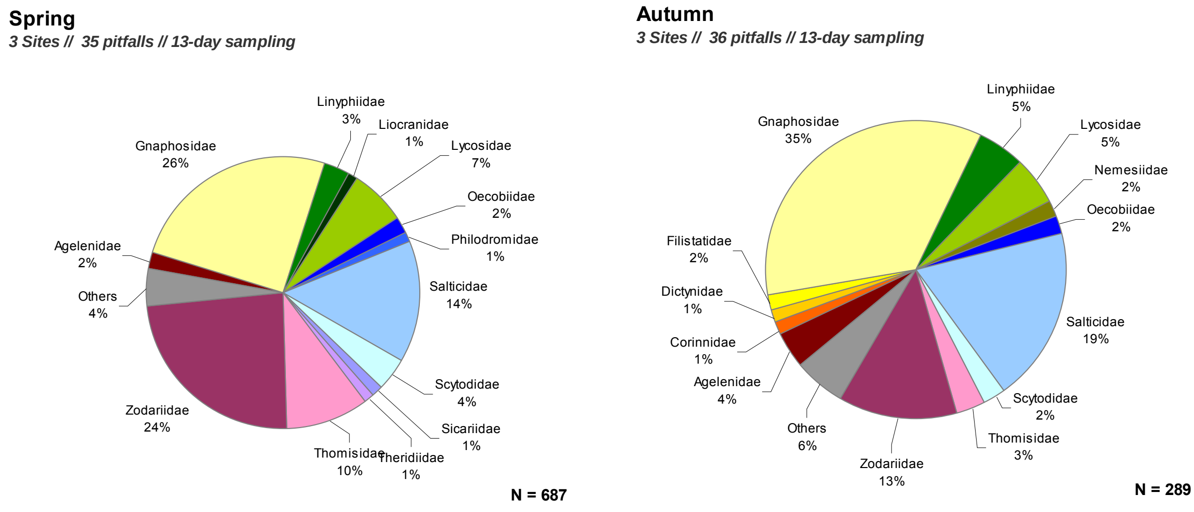

Figure 7. Shifts in families' relative abundance in Spring and Autumn in the three common sites.

Other differences can be seen between Spring and Autumn: a greater proportion of Zodariidae and Thomisidae in Spring; a greater proportion of Salticidae in Autumn. However, the overall community structure is significantly altered if we consider more than theses three sites [compare Autumn in Figures 7 and 8], so further comparisons in terms of family structure should be made in the future with the data from all Spring sites.

As for the overall Autumn community [Figure 8], Gnaphosidae being the most abundant group is in accordance with previous studies in the Mediterranean (Chatzaki et al. 1998), Portugal (Cardoso et al. 2004; Tavares 2007), and particularly in its southern region (Cardoso et al. 2007a). The second most abundant family, Zodariidae, has a relative abundance that is similar to the proportion of 13 to 45% described by Cardoso (2004) in a 10 month pitfall sampling of three biomes of the Parque Natural do Vale do Guadiana (PNVG) (approximately 30 kilometres south-east of Neves-Corvo) that are similar to those found in the study area. The high relative abundance of Linyphiidae in Autumn is also to be expected as this family has a diversity and abundance peak during the winter months in Mediterranean areas (Cardoso et al. 2007b). Also of note is the proportion of Nemesiidae, which although inflated by a site of unusually high density (50 adult males were caught in the 12 traps during the 13-day period, amounting to 45.8% of all nemesiids captured), is, in conjunction with the presence of the family Ctenizidae, expected due to Autumn being the mygalomorph mating season (Cardoso et al. 2007b).

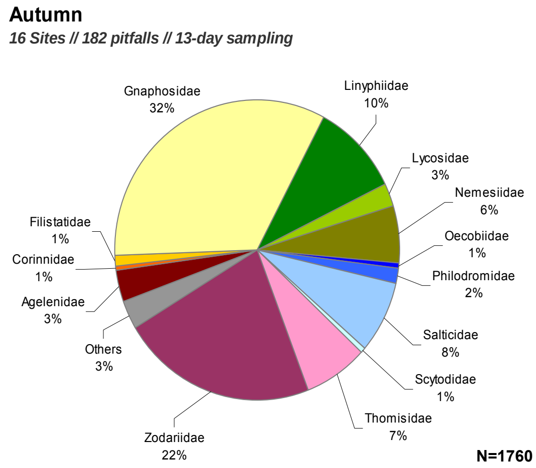

Figure 8. Autumn spider community family structure at Neves-Corvo.

Finally, the guild structure of the Autumn epigeous spider community [Figure 9] is predictably dominated by the Ground Hunters guild (with Gnaphosidae as the predominant family), followed by the Specialists (of which almost all representatives are zodariids), the Other Hunters guild (mainly Linyphiidae and Salticidae), the Ambush Hunters (most of which are thomisids of the ground-wandering Xysticus genus) and, finally, the Sheet Web weavers guilds (represented only by Agelenidae). No Orb Web weaver representatives and only three specimens of the Space Web weaver guilds were captured in the Autumn pitfalls; this is expected of a epigeous spiders oriented method, as both these guilds are characteristic of the habitat upper strata (herbaceous, shrub and arboreal).

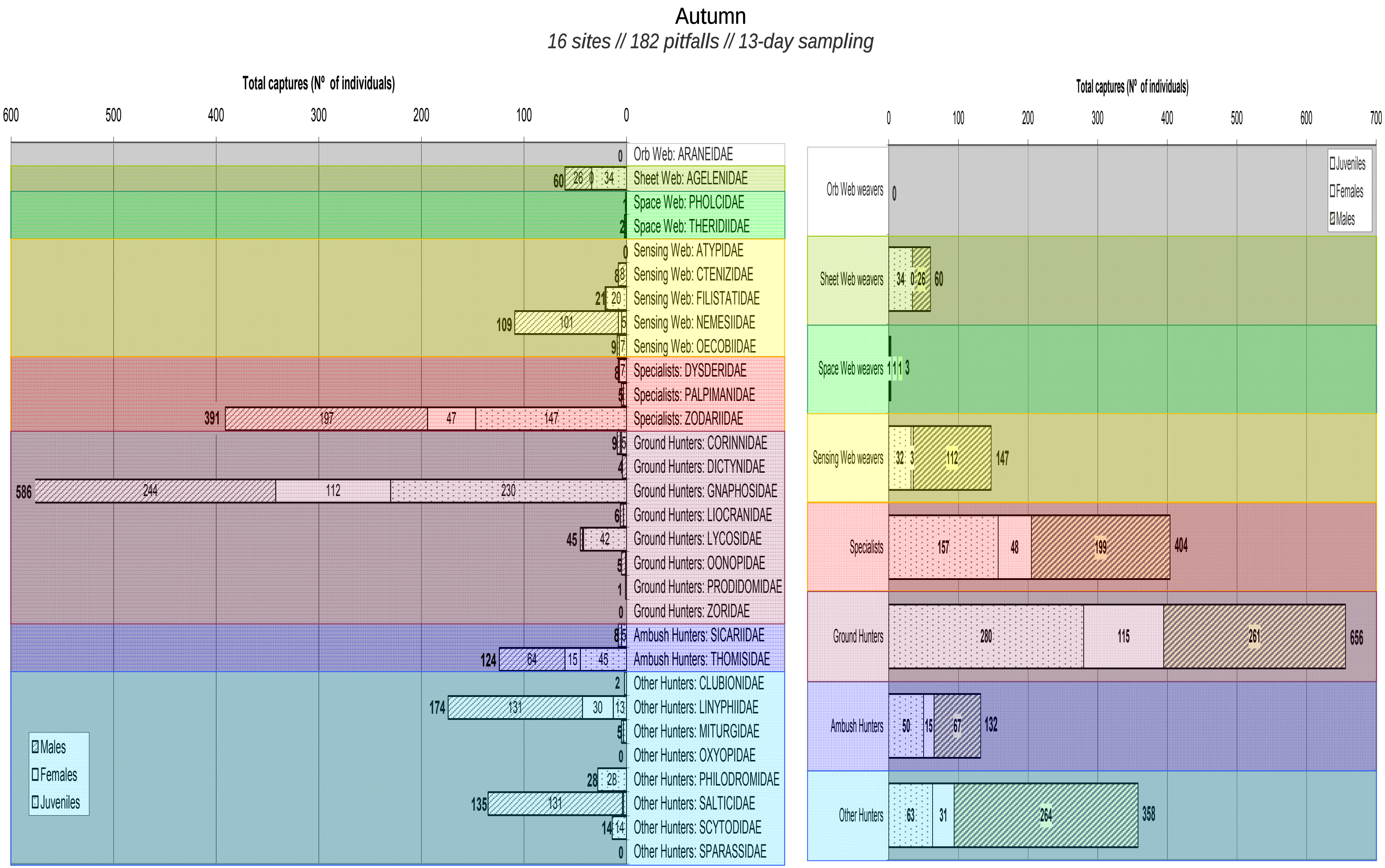

Figure 9. Total captures for the second Autumn sampling. The contribution of each guild and family, as well of each age and sex categories, to the total individuals captured is shown.

3.2. Soil and spider heavy metals contents

3.2.1. Comparison with literature values

To verify whether spider heavy metal bioaccumulation is a valid hypothesis of causation for the community variation patterns found, we analysed the Cu, Fe and Zn metal contents of spiders of the Gnaphosidae family across a spatial gradient of distance to the mine and related it to the information given by soil bioavailable contents and lichen data. The values we obtained for each of these variables can be consulted on Table 1.

Table 1. Values obtained for each of the selected study variables for each of 16 Autumn sites. Sites are ordered by increasing distance from the centre of the mine. Direction refers to the directional axis sites follow in relation to the centre of the mine. C is used for the sites close to the centre.

Site	Direction	Dist. to Mine Centre (m)	Lichens Bioavail. Cu (µg/g DW)	Lichens Particulate Cu (µg/g DW)	Soil Bioavail. Cu (µg/g DW)	Soil Bioavail. Fe (µg/g DW)	Soil Bioavail. Zn (µg/g DW)	Cu in Gnaphosidae (µg/g DW)	Fe in Gnaphosidae (µg/g DW)	Zn in Gnaphosidae (µg/g DW)	LDV
B-01	C	219.42	52.37	93.78	265.62	277.49	28.64	409.59	1053.36	528.13	4.03
B-02	C	233.08	51.35	88.53	904.14	1232.95	72.28	317.33	388.67	674.50	5.57
B-04	E	418.41	52.55	97.50	224.64	592.39	98.25	431.32	847.94	782.25	11.89
B-03	N	463.63	51.61	82.14	368.82	812.30	73.93	176.88	801.04	371.88	3.78
A-16	E	567.57	50.89	93.40	249.34	520.06	23.85	273.44	584.03	560.42	5.27
A-06	S	686.39	54.21	109.80	38.77	321.32	6.17	81.17	194.19	357.75	13.04
B-09	W	690.20	48.34	91.89	66.17	394.08	6.55	251.19	345.23	871.59	18.54
A-11	W	702.50	50.57	93.06	306.61	514.84	43.27	175.29	179.81	452.50	19.58
B-08	NE	880.17	47.30	76.20	112.27	406.62	13.41	128.18	130.63	304.15	12.46
A-05	E	1028.19	48.21	87.92	168.18	352.73	26.16	304.67	357.46	440.96	9.50
A-13	N	1066.85	45.95	72.75	20.75	471.60	5.04	110.21	116.07	378.25	7.65
A-19	NW	1073.67	43.53	75.41	34.00	216.37	2.62	289.93	361.60	485.35	21.75
B-15	S	1115.28	49.53	93.61	79.58	288.51	1.36	204.31	271.69	474.44	24.17
B-14	W	1144.69	43.35	86.90	18.14	258.64	2.82	104.69	261.07	344.32	8.24
A-07	S	1604.97	45.52	90.40	12.97	281.75	2.24	224.55	289.58	705.38	4.44
A-12	E	1966.34	42.79	78.55	24.77	310.00	4.07	169.96	238.66	436.88	16.16

The bioavailable fraction of lichen Cu contents (LBioCu) had an average value of 48.63±3.59 µg/g DW (max.: 54.21; min.: 42.79), while the non-available fraction (LPartCu) had an average value of 88.24±9.52 µg/g DW (max.: 109.80; min.: 72.75). LDV had an average value of 11.63±6.70 (max.: 24.17; min.: 3.78). Of all variables, the lichen ones were those with the narrowest intervals of variation.

In the soil, measured bioavailable Cu (SBioCu) had an average value of 180.92±225.50 µg/g DW (max.: 904.14; min.: 12.97), while Fe (SBioFe) averaged 453.23±258.19 µg/g DW (max.: 1232.95; min.: 216.37) and Zn (SBioZn) had an average value of 25.67±30.63 µg/g DW (max.:98.25; min.: 1.36).

The maximum total Cu content allowed by law in Portugal (Portaria 176/96, 1996) is 50 to 200 µg/g DW, depending on soil pH, and the maximum Zn content is 150-450 µg/g DW; Fe content is not the subject of legal regulation. Considering that total soil contents are always higher then the bioavailable fraction, with a ratio that can be on average 3.4:1 for Cu and 3.8:1 for Zn in Portuguese soils (Correia et al. 2009), many sites in Neves-Corvo have soils which are clearly above the defined limits for Cu, but well within these limits for Zn.

Of the few spider studies that included soil metals bioavailability, the only one with a comparable degree of Cu contamination is that of Hunter et al. (1987b), which described a concentration of Cu extracted by EDTA of 5800± 1670 µg/g DW for the site nearest to a Cd-Cu refinery, 268±52.5 µg/g DW for the site 1 km away from the refinery and 5.3± 0.3 µg/g DW for their control site. In this study, significant differences were detected between Cu metal contents of spiders in the contaminated sites and the control. As for Zn, there are both studies with higher values of extractable Zn, of approximately 500-700 µg/g DW (Hendrickx et al. 2004), and lower, with a value of 3.2±0.43 (Straalen et al. 2001) at their more contaminated sites; neither of these studies showed significant correlation between Zn metal contents in spiders and soil bioavailability. Fe, being a rarely studied metal in the context of impact assessment studies, has only been described in one study with its bioavailable fraction, with a value of 15.1± 2.4 µg/g DW at the most contaminated site (Straalen et al. 2001), which is much lower than the minimum value detected at Neves-Corvo.

Spider heavy metal Cu contents (<u>GnaphoCu</u>) were in average 228.89±104.74 µg/g DW (max.: 431.32; min.: 81.17), Fe (<u>GnaphoFe</u>) were 401.31±276.48 µg Fe/g DW (max.:1053.36; min.: 116.07) and Zn (<u>GnaphoZn</u>) were 510.55±166.87 µg Zn/g DW (max.: 871.59; min.: 304.15). These results were obtained from pooled samples with different proportions of juvenile, female and male individuals. Because, as mentioned previously, there can be age and sex-differences in contents, we tested for significant correlations between obtained values and the proportion of juveniles, females and males, and found only a significant correlation for Zn [see Annex II, Table 1].

The higher Cu metal contents detected in spiders in our studies are within the values described in several other studies , and are higher than those described for two species of gnaphosids (max.:314.6; min.:77.3 µg/g DW) by Rabitsch (1995). Zn spider contents for Gnaphosidae were comparable to those found for *Pirata piraticus* (Lycosidae) by Hendrickx et al. (2004) and slightly lower than those found previously in gnaphosids (max.: 1552; min: 643 µg/g DW) (Rabitsch 1995). Fe contents in spiders have only been described twice, and only once for spiders of a contaminated site (Hopkin and Martin 1985): the average value found was 416.06±69.25 µg/g DW.

To our knowledge, this is only the second study where metal analysis is performed on gnaphosids (Rabitsch 1995). In ecological studies, whether specimens of the same family are pooled together or a representative species is chosen, Lycosidae is the most commonly used family, followed by Linyphiidae (Hunter et al. 1987b). This reflects the fact that most heavy metal studies are made in temperate ecosystems of the northern hemisphere, where Linyphiidae is the most diverse and abundant family year-round (Cardoso et al. 2007b), and where Lycosidae is one of the most abundant of the ground-hunting families, as well as being one that more easily provides the necessary biomass even in heavily polluted sites (e.g. Hunter et al. 1987b; Laing et al. 2002). However, in a Mediterranean context, Gnaphosidae may present a better subject for heavy-metal contents studies, not only because they are the most abundant and species rich family in these ecosystems (see Section 3.1), but also because they are very unlikely aeronauts (Dean and Sterling 1985; Greenstone et al. 1987). Although no mention of it is made in heavy-metal studies using Linyphiidae and Lycosidae, it is possible that ballooning phenomena may be an important source of dilution of site-contamination effects: more so for Linyphiidae, the principal ballooning family (Pearce et al. 2005), as ballooning sometimes occurs en masse (Foelix 1996) and even in adults; than for Lycosidae, which become less prone to ballooning as they grow (Greenstone et al. 1987). A rate of as many as 14.8 spiders/m2 per day has been detected in a crop system during a peak period of dispersal, 7.0 spiders/m2 per day in a non-crop one (Pearce et al. 2005): numbers which may influence heavy metal content analysis done during peak seasons.

The great species richness of Gnaphosidae also means that by using a family approach, we are averaging the impact that contamination can have on a number of potentially very different physiologies, which, although obscuring opposite-direction responses, allows us to get a broader sense of the overall contamination that can occur at family-level.

3.2.2. From where does contamination originate?

If the mine is indeed the source of Cu, Fe and Cu particulate pollution, it is expected that a strong gradient with distance to the mine can be observed and that increased metal contents in lichens, soil and spiders can be significantly explained to this source of contamination.

Lichen data has a logarithmic relation with distance to the mine, significant only for LBioCu (R^2=0.593; Figure 10) and LDV (R^2=0.275; graph not shown), implying that both descriptors are influenced by the mine. A more throughout description of the gradients with distance for lichen bioavailable and particulate contents, as well as LDV can be found in Branquinho et al. (1999).

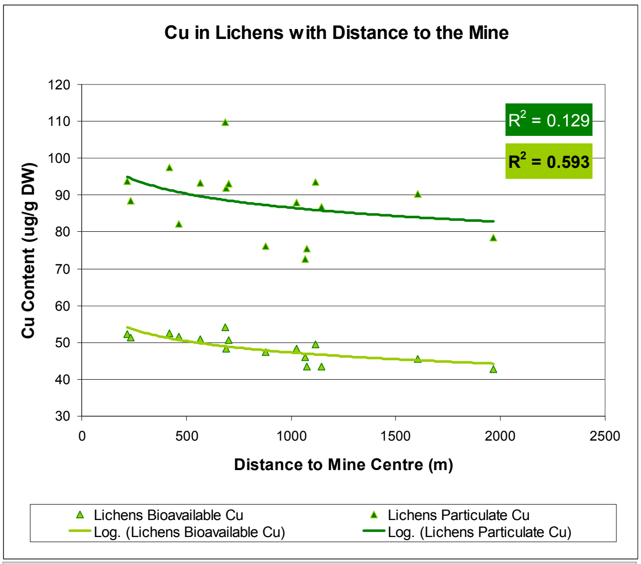

Figure 10. Graphic with logarythmic models fo LBioCu and LPartCu with distance to the mine.

An exponential relation was found between soil available metal contents and distance to the mine, in agreement with the findings of Hunter et al. (1987a). Additionally, for all three studied metals, distance was found to significantly explain variation [Figure 11a]. Of these three, however, it is SBioCu that shows the strongest affinity with distance, followed by SBioZinc. SBioFe shows a gradient of distance to the mine, but it is the weakest, with only 30% of variation explained. This is expected if we consider that Fe can have many sources, including exposed land (due to agriculture or construction), unpaved roads, and other diffuse sources (Pinho et al. 2008); and although some of these miscellaneous sources can be said to exist with higher intensity near the mine (namely, dust, roads and construction), they also occupy a much broader area, diffusing the gradient.

The relation of spider heavy metal contents with distance was best described by a logarithmic function. In Figure 11b, we can see that both GnaphoCu and GnaphoFe have statistical significant relations, but not GnaphoZn. Additionally, for spider contents, unlike what happens with soil bioavailable contents, the strongest gradient with distance is for GnaphoFe, the weakest of the three soil metal gradients with distance.

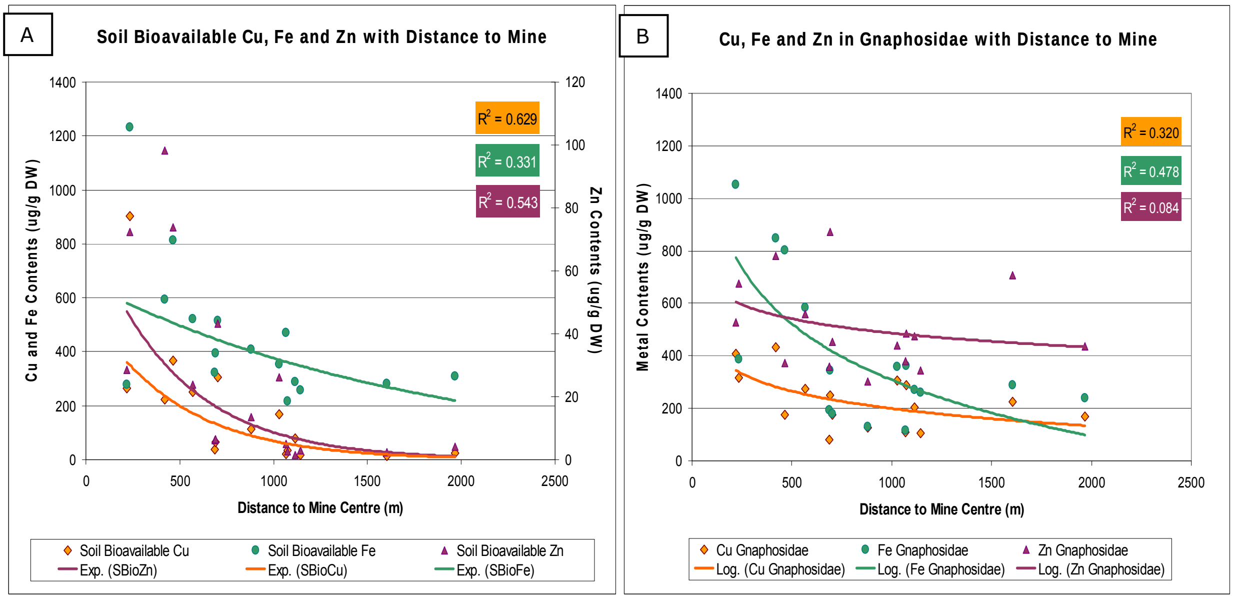

Figure 11. Biplots of Cu, Fe and Zn contents in soil (A) and in Gnaphosidae (B) with distance to the mine centre. Relations for soil are exponential and for spiders are logarithmic. R-square values for each bivariate model are shown.

Next in terms of strength of the gradient, is GnaphoCu, which we would have expected to be the strongest based on soil results. However, physiological studies on the effects of Cu in spiders have revealed that Cu can increase cellular levels of Fe (Wilczek et al. 2004). This then could explain why GnaphoFe shows the strongest gradient with distance.

GnaphoZn shows no gradient with distance to the mine, despite the strong gradient that exists for SBioZn. Two things might contribute to this: 1) the low amount of Zn in the soils, which might not be enough to be a significant source of contamination; 2) the influence of sex in the measurement of Zn contents [Annex II, Table 1]. Although the first hypothesis seems to be supported by the lack of significant bioaccumulation of Zn when higher contents of the metal are bioavailable in the soils (Hendrickx et al. 2004), such evidence must be taken with due caution as we are comparing different families and systems.

A third possibility is suggested by the enrichment values found closer to the mine for both soil and spider contents [Figure 12]. Despite the relatively low values of SBioZn registered, the magnitude of the difference between these values is extremely high, resulting in an enrichment that is at the same level of that of SBioCu. Looking at the enrichment values for spiders, we find that they are much smaller than those found in soils. This then, could be evidence of the existence of very efficient physiological regulation mechanisms in spiders for both Cu and Zn. The high end of the curves for GnaphoCu and GnaphoZn in Figure 11b also seem to support this, in that most of the metal contents observed in spiders seem to be intrinsic to the spiders themselves, unlike what happens with the curve for GnaphoFe, which ends in a sudden drop.

A high efficiency regulation mechanism for Cu has also been suggested by the data of Hunter et al. (1987b). Such tight regulation might indeed be expected for spiders, as their respiratory pigment, hemocyanin, is a copper-containing protein (Foelix 1996).

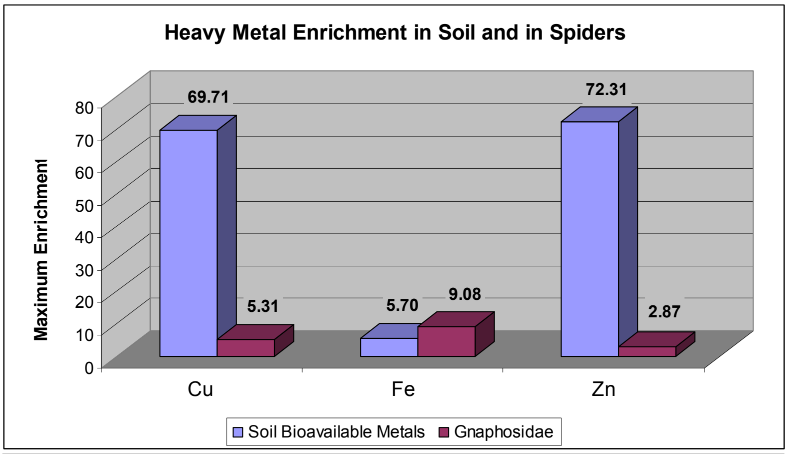

Figure 12. Cu, Fe and Zn enrichment closer to the mine, for both soil and spider contents.

As for Fe, the fact that enrichment in spiders is superior to that of soils could suggest bioaccumulation; however, the evidence of such a strong gradient with the mine rather suggests that an active incorporation of Fe in the cells as a physiological relief mechanism of the effects of toxicity could be taking place. The subject clearly merits further research to elucidate the question.

In conclusion, a strong gradient with the mine can indeed be observed for most of the studied parameters, supporting the hypothesis that the mine is an important source of contamination, through aerial deposition, of the soils. However, this increase in metals in the environment is not markedly manifested in higher metal contents in spiders, probably due to efficient regulation.

3.2.3. What is the source of the higher spider heavy metal contents?

In order to ascertain the source of heavy metal contents in spiders we used lichens and soil variables, as well as with distance, to perform Spearman correlations [Table 3].

Table 3. Spearman correlations between distance, lichen and soil variables and spider heavy metal contents. Significant values at $p<0.05$ are in blue; relations above $r=0.60$ are in bold.

Spearman R	Gnaphosidae Cu	Gnaphosidae Fe	Gnaphosidae Zn
Distance to Mine Centre	-0.459	-0.509	-0.266
Lichens Bioavailable Cu	0.384	0.478	0.239
Lichens Particulate Cu	0.442	0.575	0.475
Soil Bioavailable Cu	0.566	0.531	0.281
Soil Bioavailable Fe	0.113	0.002	0.078
Soil Bioavailable Zn	0.419	0.393	0.168
LDV	**-0.795**	**-0.736**	**-0.610**

GnaphoCu is negatively and significantly correlated with Distance ($r=0.566$) and positively with SBioCu ($r=0.459$); there is no significant correlation with LBioCu or LPartCu. This suggests that Cu in spiders reflects soil contamination directly, and aerial deposition of contamination only indirectly.

GnaphoFe, on the other hand, is negatively correlated with Distance ($r=-0.509$) and positively correlated to LBioCu, LPartCu and SBioCu ($r>0.470$), but not SBioFe or SBioZn. Fe in Gnaphosidae seems therefore to be the metal in spider contents that is better correlated with the contamination parameters most affected by the mine, as it reflects both aerial and soil contamination.

Zn is the only metal is Gnaphosidae not significantly correlated with distance. It is also significantly correlated with LPartCu, but an explanation for this latter correlation is lacking.

Interestingly, the only correlations with environmental assessment variables with $r>0.60$ are, for all three metals in Gnaphosidae, with LDV; this suggests that, within the 4km diameter area of the study site, both biological indicators reflect the conditions that propitiate contamination of the biota.

3.3. Variations in spider abundance and community structure across a contamination gradient

3.3.1. Abundance

- ***Overall ground-spiders community***

As we have seen in Section 3.2.2., despite the high enrichment values found for Cu and Zn in the soils closer to the mine, a concomitant increase in heavy metal contents in Gnaphosidae was not observed, probably due to physiological regulation. This regulation, however, comes at a cost: spiders have to allocate energy to these detoxification mechanisms that would otherwise allocate for growth and reproduction (Hendrickx et al. 2003c). Additionally, as other arthropods might not be as successful as spiders in regulating these metals (Hunter et al. 1987b; Spurgeon et al. 1996), a decrease in the number of prey might occur (Bengtsson and Rundgren 1984). For these two reasons, a significant decrease in spider abundance in sites where contamination values are higher is expected.

The r values of the bivariate models for spider community overall abundance confirms these expectations [Table 4], with a significant and high positive response to Distance (r=0.677) and significant and high negative responses (r<-0.60) being found for LBioCu, LPartCu, SBioCu, SBioZn and GnaphosFe: the contamination variables which have the strongest gradient with distance to the mine.

Table 4. Overall spider abundance model fitting r-values. Significant values at p<0.05 are in black. Values above 0.60 are in bold.

r	Variable:	Distance	LBioCu	LPartCu	SBioCu	SBioFe	SBioZn	GnaphoCu	GnaphoFe	GnaphoZn	LDV
N	Type of model:	Log.	Linear	Linear	Log.	Log.	Log.	Linear	Linear	Linear	Linear
617	Juveniles	**0.623**	-0.304	-0.083	**-0.638**	-0.529	**-0.627**	-0.403	-0.589	-0.308	0.271
213	Females	**0.633**	**-0.665**	-0.318	**-0.663**	-0.424	**-0.638**	-0.375	**-0.672**	-0.059	0.538
930	Males	0.370	-0.588	**-0.620**	-0.291	-0.110	-0.303	-0.467	**-0.602**	-0.533	0.546
1143	Adults	0.466	**-0.653**	-0.590	-0.409	-0.200	-0.412	-0.480	**-0.666**	-0.452	0.586
1760	All	**0.677**	**-0.618**	-0.447	**-0.647**	-0.443	**-0.643**	-0.559	**-0.793**	-0.485	0.553

The question of whether it is the high affinity with the mine that causes these relations to appear, and not necessarily heavy metal pollution is moot: distance is a variable that unifies all the impacts of the mine, including contamination. However, if it were other anthropogenic impacts that drove the observed explainability, it would be expected that distance to the mine would explain markedly more than the contamination variables, but this does not occur; the explainability of distance to the mine seems to be essentially the explainability of mine-originated contamination.

It is also of note how Cu, Fe and Zn contents in Gnaphosidae, despite referring to only one family, albeit the most abundant in the community, can have very high r-values for the overall community (higher even than those for the Gnaphosidae or the Ground Hunters; Annex II, Table 2), perhaps reflecting a community-level tendency for abundance to decrease with increasing potential for bioaccumulation of metals.

An increase in spider abundance at the most polluted sites, as was found by Spurgeon et al. (1996), was not observed. Instead, our results corroborate the findings of Bengtsson and Rundgren (1984), who described a significant reduction in the abundance of all studied arthropods, including spiders, near a brass mill at Gusum. It would be interesting to explore whether the differences between the study of Spurgeon et al. (1996) and the similarities with that of (Bengtsson and Rundgren 1984) could be due to differences in soil metal availability. Unfortunately, only total metal values are available for both studies, so comparisons are not possible; the differences between the perceived pollution in total soil contents and in availability can be great, as demonstrated by Straalen et al. (2001). The use of total values might give an erroneous idea of the risk heavy metals may pose to the biota (Kabata-Pendias 2004), and it is possible that sites with very high total values will have no measurable impact on the spiders, because the bioavailable fraction in not correspondingly high.

Juveniles, females and males

How different would be the perceived patterns of spider community abundance if we only included adults in the analysis? Firstly, the explainability of most relations would decrease, and only GnaphoFe and LBioCu would remain above the r=0.60 threshold. Soil bioavailable metals contents would also cease to be significant variables (r<0.426). Conclusions are therefore hardly the same, as ground-spider communities would then be found to be irresponsive to soil contamination.

In Table 4 we can see that this pattern is mostly due to the abundance pattern of males, while female abundance follows the overall community pattern we have previously described. But because females are undersampled in pitfalls, the weight of the 213 individuals captured is almost completely obscured by the 930 males. Juveniles, however, are caught in greater numbers (N=617), and observe a similar pattern of abundance to that of females; the most significant difference between females and juveniles is that the latter do not seem to respond directly to the atmospheric pollution (LBioCu=-0.304; LPartCu=-0.083) or have a relation with LDV (r=0.271). The inclusion of juveniles, however, is suggested by the data as being vital to the perception of overall community abundance patterns, particularly when pitfalls are the sampling method used. This is in accordance to the conclusions of Jiménez-Valverde and Lobo (2006), who identified to the species juveniles of two families where such an identification was possible and compared the patterns of richness and abundance including and excluding juveniles.

But why are the patterns for juveniles, females and adults distinct? One of the reasons has already been discussed, and it is the hypothesis that juveniles and adults have different sensibilities to heavy metals and perhaps different requirements in terms of prey (small juveniles often feed on smaller prey, like the Collembola), resulting in different patterns. The other hypothesis is that differences in representativity of species in the adult and juvenile populations (abundance patterns not always being synchronous; Norris 1999) are responsible for the differences in the patterns, in which case including juveniles permits the inclusion of species that are present in the community at the moment, but haven’t yet reached maturity.

An additional source of difference is mobility and behaviour in juveniles, females and males. Juveniles, after their second molt, actively disperse and, as we have seen, have the highest tendency for ballooning. Dispersal to other sites where hopefully they can find better conditions could therefore a confounding factor; however, more directly, and especially for the less vagile families, their number, as said, is a function of the female reproductive output, and also, on the availability of food that will permit their continued survival: it is perhaps because of this that juvenile abundance patterns seem to most closely resemble female ones.

Once they reach maturity, males are extremely active, as their lives are short and they have a small window to maximise their successful encounters with the opposite sex. They are known to even forego eating while in search for a mate (Foelix 1996). It is possible then, that the abundance pattern for males may be more erratic, and less site specific perhaps even than that of juveniles.

Females are out of the three components the most site-faithful as, although they move about as well, they are not as active (evidenced by the fact that they are rarer catches in pitfalls). They also generally live longer than the males and are more likely to stay at site where there is abundant prey and relocate when there is shortage (Wise 1993).

Females could be argued to be the best barometers of a localized populations' health in terms of contamination and resource abundance, as they represent the share of juveniles that survived to adulthood and that will contribute to continuity of the population. A lower number of adult females means that, despite eventual adaptive mechanisms that might contribute to a higher success rate in a poorer habitat (like larger egg size), resources are insufficient for a higher number of adults to be sustained. These resources might be prey items (which can also be much reduced in contaminated places) or resources to allocate for reproduction due to detoxification efforts.

So females would be probably be the most desirable spiders for pattern analysis, but they are normally poorly represented in pitfalls, which makes having statistically robust samples difficult. In the Autumn sampling, the only families that had enough individuals for a separate analysis were Gnaphosidae (N=112), Linyphiidae (N=30) and Zodariidae (N=47) [Annex II, Table 2].

Overall ground-spiders community abundance was found to decrease with increasing contamination and to increase with increasing distance from the mine and LDV, and is therefore suggested to be a good parameter for evaluating the impact of the mine on the epigeous spider community.

- ***Guilds***

Guilds represent groups of spiders that have similar ways of exploring their resources, and are presumed to hunt the same sub-set of a larger resource: arthropods (Uetz et al. 1999). This might result in members of different guilds to have distinct accesses to contamination, depending on what they eat (Hendrickx et al.

2004). Guilds therefore represent ecologically relevant units, and each one might respond differently to contamination.

Of the seven Autumn guilds, one was excluded from the analysis due to insufficient specimens (Space Web weavers; see Figure 9). Of the remaining six guilds, only the Ground Hunters and the Specialist guilds had high and significant relations to the studied variables [Table 5].

Table 5. Overall spider abundance model fitting r-values. Significant values at p<0.05 are in black. Values above 0.60 are in bold.

r	Variable:	Distance	LBioCu	LPartCu	SBioCu	SBioFe	SBioZn	Gnapho Cu	Gnapho Fe	Gnapho Zn	LDV
N	Type of model:	Log.	Linear	Linear	Log.	Log.	Log.	Linear	Linear	Linear	Linear
60	Sheet Web	0.096	-0.405	-0.400	-0.141	-0.252	-0.220	0.145	-0.032	0.075	0.253
147	Sensing Web	0.307	-0.097	-0.051	-0.126	-0.228	-0.479	-0.171	-0.202	-0.170	-0.007
404	Specialists	0.363	-0.559	**-0.724**	-0.326	0.029	-0.235	-0.397	-0.578	-0.318	0.503
656	Ground Hunters	**0.677**	-0.403	0.054	**-0.743**	**-0.616**	**-0.656**	-0.475	**-0.646**	-0.256	0.432
132	Ambush Hunters	0.497	-0.253	-0.078	-0.141	-0.303	-0.180	0.030	-0.203	-0.135	-0.368
358	Other Hunters	-0.187	0.113	0.205	-0.072	-0.100	0.061	-0.137	-0.057	-0.250	0.236

The Specialists guild

Specialist abundance has a significant and negative relation to LBioCu and LPartCu and a positive one to LDV, but only LPartCu has an explanability of over 40% (r>0.60). Specialists seem therefore to reflect aerial deposition, and particularly the non-available fraction of aerial deposition, rather than contamination of the soil.

The Specialist guild is, as the name implies, formed by families that are group-specific hunters. In the Autumn Neves-Corvo community, specialists are represented by families Dysderidae, which hunts woodlice (Isopoda) (Hopkin and Martin 1985); Palpimanidae, which specialises in salticid spiders (Cerveira and Jackson 2005); and Zodariidae, specialist ant-eaters (Pekár 2004). Almost all specimens captured were of this last family [Figure 9]. It is therefore possible that the pattern shown reflects some aspect of ant-abundance, or of a specific genre or species of ant, as zodariid species have differing hunting successes with different kinds of ants (Pekár et al. 2004), but it can also be due the biology of the spiders themselves.

Table 6. Model fitting r-values for specialist spider abundances. Significant values at p<0.05 are in black. Values above 0.60 are in bold.

r	Variable:	Distance	LBioCu	LPartCu	SBioCu	SBioFe	SBioZn	Gnapho Cu	Gnapho Fe	Gnapho Zn	LDV
N	Type of model:	Log.	Linear	Linear	Log.	Log.	Log.	Linear	Linear	Linear	Linear
157	Specialists: Juveniles	**0.682**	**-0.712**	-0.569	-0.566	-0.414	-0.526	-0.250	-0.561	-0.179	0.311
48	Specialists: Females	0.199	-0.405	**-0.613**	-0.279	0.181	-0.165	-0.465	-0.530	-0.340	0.558
199	Specialists: Males	0.131	-0.379	**-0.678**	-0.121	0.247	-0.026	-0.376	-0.468	-0.323	0.488
247	Specialists: Adults	0.146	-0.388	**-0.672**	-0.154	0.236	-0.055	-0.398	-0.485	-0.330	0.507
404	Specialists: All	0.363	-0.559	**-0.724**	-0.326	0.029	-0.235	-0.397	-0.578	-0.318	0.503

If the first hypothesis were true, we would expect an equally strong response from specialist juveniles and adults. However, looking at the results of the model fitting for all three components [Table 6], we can see that females and males have the same abundance pattern as that described for the overall Specialist abundance. Juveniles, however, have a very different pattern from that of adults, with a high significant negative r-value for LBioCu (r=-0.712) and a positive one for Distance (r=0.682). A difference in biology between juveniles and adults seems, therefore, more likely.

The Ground Hunters guild

The Ground Hunters guild is formed by families that hunt on the ground, actively pursuing their prey. Although seven families of the guild were captured in Autumn, only Gnaphosidae and Lycosidae had enough specimens for and individual analysis. Patterns at the guild level [Table 7], however, echo the patterns found for Gnaphosidae [Annex II, Table 2].

Table 7. Model fitting r-values for ground hunter spider abundances. Significant values at p<0.05 are in black. Values above 0.60 are in bold.

r	Variable:	Distance	LBioCu	LPartCu	SBioCu	SBioFe	SBioZn	Gnapho Cu	Gnapho Fe	Gnapho Zn	LDV
N	Type of model:	Log.	Linear	Linear	Log.	Log.	Log.	Linear	Linear	Linear	Linear
280	Ground Hunters: Juveniles	0.221	0.247	0.474	-0.326	-0.353	-0.345	-0.260	-0.289	-0.179	0.007
115	Ground Hunters: Females	**0.610**	**-0.634**	-0.091	**-0.601**	-0.553	-0.585	-0.248	-0.436	0.117	0.411
261	Ground Hunters: Males	0.509	**-0.717**	-0.567	-0.472	-0.236	-0.300	-0.302	-0.451	-0.250	0.513
376	Ground Hunters: Adults	0.657	**-0.827**	-0.473	-0.625	-0.423	-0.486	-0.340	-0.537	-0.139	0.573
656	Ground Hunters: All	**0.677**	-0.403	0.054	**-0.743**	**-0.616**	**-0.656**	-0.475	**-0.646**	-0.256	0.432

Variation in abundance of the Ground Hunter guild is highly and significantly explained by with soil contamination variables: SBioCu being the one with the highest r-value (r=-0.743), followed by SBioZn (r=-0.656) and SBioFe (r=-616). Ground Hunter abundance also increases significantly with distance to the mine (r=0.677) and with decreasing GnaphoFe (r=-0.646).

This pattern is distinctively different from that of Specialists, which were better associated with the aerial deposition and contamination; Ground Hunters instead respond negatively to all aspects of soil contamination, as well as to the increasing concentrations of Fe found in their principal representatives, the Gnaphosidae. This would suggest a dichotomy of bioindication in the mine: Specialists would reflect the aerial aspect of the mine's impact, while Gnaphosidae the direct repercussions of this air-borne deposition on the soil.

However, like what happens in the Specialists guild, Ground Hunter juveniles, females and males present different patterns of variation than that which can be observed in the guild as a whole.

Female and male Ground Hunter abundance share the same pattern: one mostly dictated by a significant and high negative relation with LBioCu (r=-0.634 and r=-0.717, respectively); a relation which gains in explainability when adults are considered together (r=-0.827). Female abundance also respond well to distance to the mine (r=0.610) and to SBioCu (-0.601), showing a higher affinity for soil contamination than males, perhaps due to their greater site-faithfulness, as explained previously.

As for Ground Hunter juveniles, abundance shows no definitive pattern with the studied variables. Despite this, explainability for Ground Hunter abundance patterns is raised when juveniles are added. For this guild, the pattern of the whole is not the pattern of its individual components. It is of note that female abundance (here obtained from the largest number of females in the whole dataset) is the one that seems to respond more closely to the overall abundance pattern, which does not disagree with the arguments presented previously, that females might be those that better reflect the impact patterns.

Still, it is positive for the future use of the Ground Hunters guild in soil contamination impact assessment studies that its abundance variation presents the best relations with SBioCu, SBioFe and SBioZn observed in the present study.

3.3.2. Community guild structure

In order to perceive possible shifts in community guild structure, we made a PCA using the proportions of each guild per site as active variables and distance, habitat complexity (HSCS) and contamination parameters supplementary variables [Figure 13].

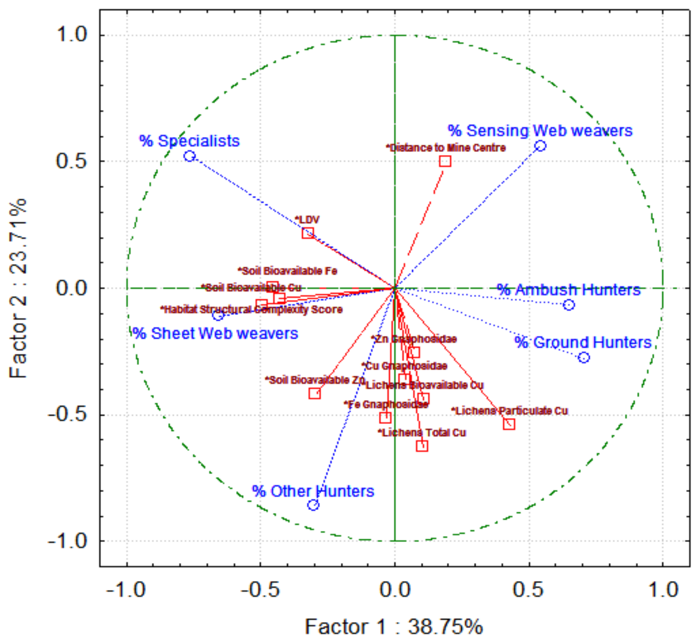

Figure 13. PCA showing the shifts in guild community structure in relation to one another (blue=active variables), and in relation to the various studied variables (red=supplementary variables).

The use of the various categories of land use and vegetation were used as grouping variables, but no pattern that echoed that of the PCA was observed; land use and vegetation were therefore concluded to have very weak, if any, confounding effects on the relative abundance patterns.

The pattern in the PCA is not unlike what was found in the abundance bivariate model analysis.

Along the first axis, increasing proportions of Ground Hunters and Ambush Hunters in the positive side of the axis, are opposed to growing proportions of Specialists and Sheet Web weavers. This first factor is accompanied by increasing levels of LPartCu in its positive side, which explains why there is a decrease in Specialists, as their abundance has been found to be sensitive to this form of pollution. Factor 1 is also accompanied by decreasing levels of SBioCu and SBioZn in its positive side, which seeing as Ground Hunter abundance responds most strongly to soil contamination, explains why the proportion of Ground Hunters increases along the axis.

An increase of the HSCS along the negative side of Factor 1 might explain the higher proportions of Sheet Web weavers, as they need structures on which to build their webs. Only by use of the integrated score and only in this particular was there a pattern with habitat structure to be discerned.

The second axis is characterized by a strong decrease in the proportion of Other Hunters. This decrease is accompanies by a decrease in contamination variables such as GnaphoFe, LPartCu, LBioCu, SBioZn, which means that the proportion of Other Hunters decreases with increasing distance from the mine. This positive association of the Other Hunters guild with the mine is probably due to the weak but significant ($r>0.426$) positive relation of family Linyphiidae (one of its most abundant representative families) with LBioCu and LPartCu [Annex II, Table 2].

Sensing Web weavers occupy an intermediate position with both factors, but an opposing relation to increasing SBioZn can be seen. This association is probably due to the fact that the site where most Sensing Web weavers was found (site B-15, characterized by an unusually high density of nemesiids), was also the site with the lowest registered value for SBioZn. As the guild was otherwise not very abundant, this resulted even in a significant r-value in the model fittings [Annex II, Table 2]; however, once B-15 is removed the significance disappears.

3.4. Evaluating contamination with soil and spider data

One of the fundamental uses of bioindication is to supply managers, stakeholders and decision-makers with biologically pertinent information about the health and integrity of ecosystems under their guard. Bioindicators have to be able to give practical information that can help establish conservation or remediation priorities.

It is important to understand how the soil and spider abundance and metal contents data would qualitatively classify the sites where sampling was undertaken and the degree of consensus between the various measures.

Previous bioindication works with lichens (Branquinho et al. 1999; Branquinho and Pinho 2005; 2006) established that at 2 km from the mine, there were no significant negative impacts on lichens.

Plotting soil and spider metal contents data with distance [Figure 14], allows us to see that by 1500-2000 meters from the centre of the mine, Cu and Zn content values for both soils and spiders seem to have stabilised, so contamination caused to the mine seems to have ceased to have an effect; this concurs with lichen data.

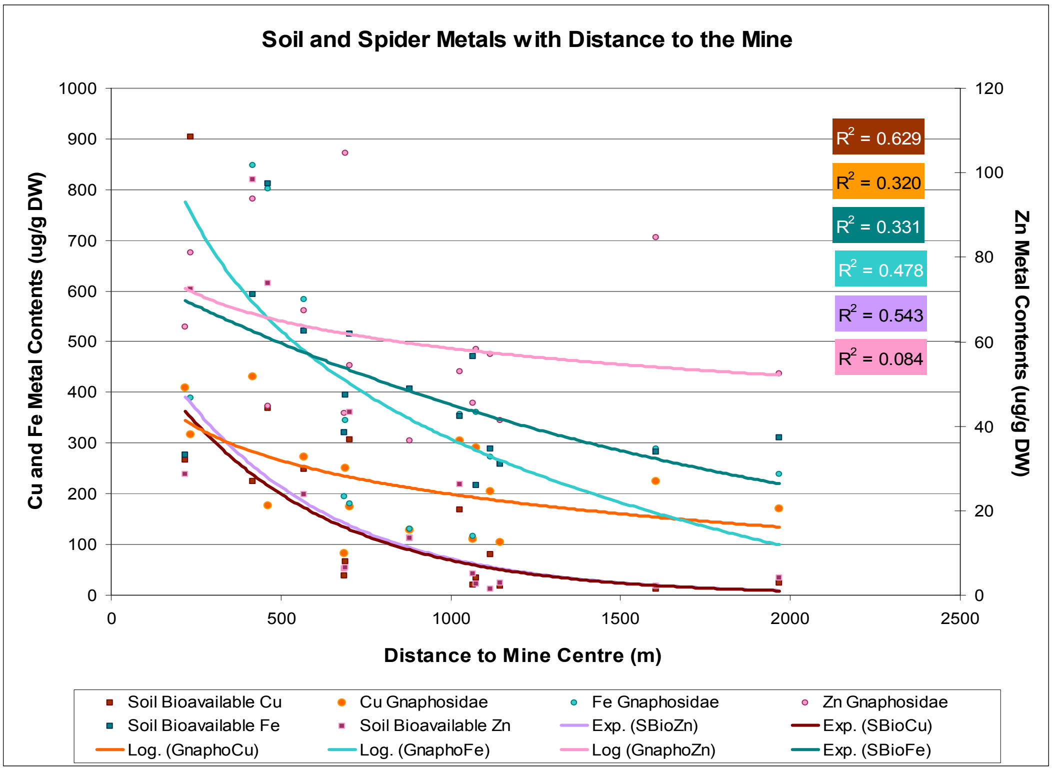

Figure 14. Biplot of Cu, Fe and Zn contents in soil and in Gnaphosidae with distance to the mine centre. Relations for soil are exponential and for spiders are logarithmic. R-square values for each bivariate model are shown.

However, we must integrate the information given by the metal contents data to that given by variations in ground-spiders community abundance: an easy-to-measure parameter found to be related to variation in contamination variables, distance to the mine and habitat quality (LDV). A score integrating the three site rankings for each of these variables will enable the discrimination between 4 classes of relative quality (from Worse to Best) [Table 8]. The resulting classification can be seen superimposed over a photo of the mine in Figure 15.

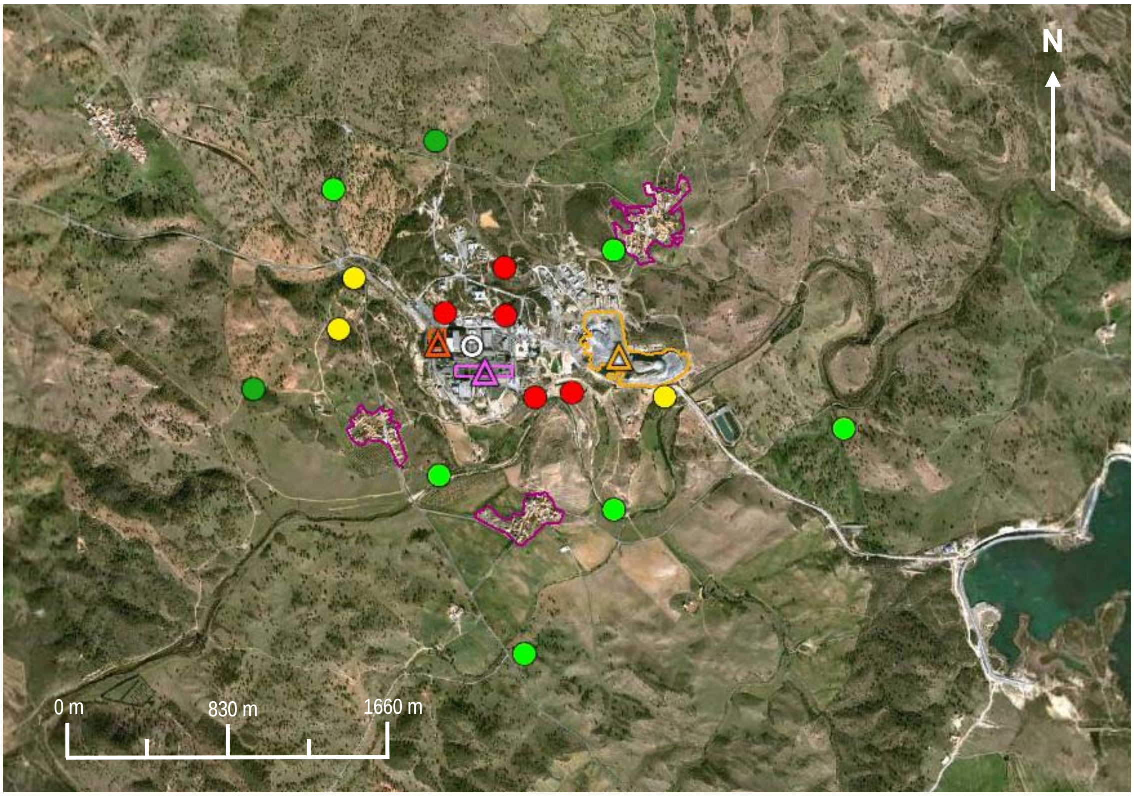

Figure 15. GoogleEarth© photo of the study area with sites marked according to the relative evaluation given by the Integrated Score of site rankings. Red – Worse quality; Yellow – Lower intermediate quality; Light Green – Higher Intermediate quality; Dark Green – Best quality.

Table 8. Sampling site ranking according to: Ground-spider abundance; Spider metal contents; Soil metal contents; Integrated Score of all three. Entries are coloured according to the quality category given by the Integrated Score: Red Red – Worse quality; Yellow – Lower intermediate quality; Light Green – Higher Intermediate quality; Dark Green – Best quality.

Ground-spiders abundance Ranking		Spider metal contents Ranking		Soil metal contents Ranking		Integrated Score	
Site	Score	Site	Score	Site	Score	Site	Score
B-03	*75*	B-04	*75*	B-02	*75*	B-04	*275*
B-01	*100*	B-01	*100*	B-03	*75*	B-02	*325*
B-04	*100*	B-02	*100*	B-04	*100*	B-03	*350*
A-16	*125*	B-09	*125*	A-11	*100*	B-01	*375*
B-02	*150*	A-16	*125*	A-16	*125*	A-16	*375*
B-09	*150*	A-05	*150*	B-08	*150*	B-09	*475*
A-05	*175*	A-07	*150*	B-01	*175*	A-05	*500*
A-06	*200*	A-19	*150*	A-05	*175*	A-11	*575*
A-07	*200*	B-03	*200*	B-09	*200*	A-07	*650*
B-08	*225*	B-15	*200*	A-06	*225*	B-08	*675*
A-11	*225*	A-03	*225*	A-13	*225*	B-15	*675*
B-14	*225*	A-11	*250*	A-03	*250*	A-19	*700*
B-15	*225*	A-13	*275*	B-15	*250*	A-06	*725*
A-03	*250*	B-14	*275*	A-19	*275*	A-03	*725*
A-19	*275*	A-06	*300*	A-07	*300*	A-13	*800*
A-13	*300*	B-08	*300*	B-14	*300*	B-14	*800*

Rankings for each of the three parameters are remarkably similar in their agreement over the “Worse quality” sites; higher variability can be observed in the intermediate, and even “Best” categories.

If not for the placement of site B-03 in the Spider Metal Contents Ranking, the information of Spider Abundance and Metals could be considered to be very consistent. However, the metal contents for site B-03 might misrepresent the *de facto* metal contents of spiders of that site, as the pooled sample from which it was obtained was the only one where only juveniles were used, and these juveniles were very small, and so probably very young, which means they might not have been as exposed to contamination as specimens used in other samples were.

The Integrated Score has some interesting classifications, when we look at the spatial arrangement of sites [Figure 15], as greater distances from the mine going east and south do not achieve the same levels of spider and soil indicated quality as those located in the north and west. This is consistent with the dominant winds directionality (NW-W to SE-E) and with results obtained for lichens (Branquinho and Pinho 2005).

4. Final considerations: the potential of bioindication with spiders

Despite the limited time-frame of the data presented here, the results of the present study indicate that spiders can be adequate bioindicators of soil contamination in the context of primarily Cu gradient in the Mediterraean climate.

- The use of pooled samples of individuals from the Gnaphosidae family allowed us to detect a gradient with distance to the mine, and significant correlations between these values and those of other parameters of contamination such as lichens and soil bioavailable metal contents were observed.
- Our study also agrees with previous findings that Cu accumulation is regulated in spiders. As for Zn, despite the substantial gradient observed, concentrations in the study area were perhaps to low for there to be any measurable effects on spiders. Fe proved to mark the distance gradient most interestingly, despite the absence of a corresponding soil gradient, a fact that can be attributed to raised levels of Fe in response to Cu. To our knowledge, this is the first time that raised Fe contents in spiders are reported in the context of a field evaluation of soil contamination; it deserves future research.
- Abundance of the ground-dwelling spider community decreased with increased values of aerial, soil and spider heavy metal contents and increased with growing distance from the mine. The abundance of different guilds showed distinct patterns of overall variation: Ground Hunters had higher affinity for soil parameters and Specialists for atmospheric deposition.
- The use of pitfalls in a spatial arrangement of sites allowed us, despite all sources of variation related with vegetation and land use, to detect strong gradients of decreasing abundance with both distance and contamination variables. The known bias of pitfalls (which we partially accounted for by discriminating between sexes and maturity) does not preclude the use of the method for relative comparisons in studies of this aimed at the community of ground-spiders. The amount of material caught in pitfalls can, however, be overwhelming. To process it in the amount of time that is given for impact assessment, identification at a family-level might be all that is feasible. Identifications at such a level have the advantage of specialist expertise not being needed beyond perhaps an initial and short phase of training; and the advantage that juveniles can be used. In this context, guilds might be a good approach to take these family-based data to an ecological level that allows for great explainability.
- The inclusion of juveniles permits the observation of more generalized and more robust patterns for the whole community, and we believe this to be particularly useful at the guild and community levels, particularly in impact assessment studies.

As for recommendations for future studies in the area, we advise the realization of at least a year-round study so juvenile-adult dynamics and seasonal shifts in guilds and families in the context of impact assessment can be properly evaluated.

Targeting females in impact assessment and ecological studies, as has been done with very interesting results in studies of life history and mate-choice, is also a subject of future consideration.

More essential research on the metabolism of Cu and Fe, especially in light of new technologies, is needed. The increased levels of Fe in spiders in response to cellular increases in Cu should be the object of further study, as like the recent studies of biomarkers, it has the potential of all be a bioindicator of contamination by Cu, when otherwise effects of this metal might be hard to detect due to efficient regulation.

We also make the general recommendation for studies on soil contamination to include a measure of metal bioavailability of the soils, particularly when a biological component is also being studied, so more accurate comparisons can be made.

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6. Bibliography

Bengtsson G, Rundgren S (1984) Ground-living invertebrates in metal-polluted forest soils. Ambio 13:29-33.

Bengtsson G, Tranvik L (1989) Critical metal concentrations for forest soil invertebrates: a review of limitations. Water, Air, and Soil Pollution 47:381-417.

Bishop L, Riechert SE (1990) Spider colonization of agroecosystems: mode and source. Environmental Entomology 19:1738-1745.

Branquinho C, Catarino F, Brown DH, Pereira MJ, Soares A (1999) Improving the use of lichens as biomonitors of atmospheric metal pollution. The Science of The Total Environment 232:67-77.

Branquinho C, Pinho P (2006) Avaliação da Qualidade do Ar na zona da mina de Neves-Corvo através da avaliação dos poluentes acumulados em líquenes (2006) - Relatório Preliminar. Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal.

Branquinho C, Pinho P (2005) Avaliação da Qualidade do Ar na zona da mina de Neves-Corvo através da utilização da biodiversidade de líquenes (2005). Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal.

Branquinho C, Serrano HC, Pinto MJ, Martins-Loução MA (2007) Revisiting the plant hyperaccumulation criteria to rare plants and earth abundant elements. Environmental Pollution 146:437-43.

Butovsky RO (1996) Insects in bioindication of soil pollution. In: Straalen NMV, Krivolutsky DA (eds) Bioindicator Systems for Soil Pollution. Springer, pp 155-162.

Cardoso P (2008) Biodiversity and conservation of Iberian spiders: past, present and future. Boletín Sociedad Entomológica Aragonesa 42:487-492.

Cardoso P (2004) The use of Arachnids (Class Arachnida) in biodiversity evaluation and monitoring of Natural Areas. PhD Thesis:1-172.

Cardoso P, Gaspar C, Pereira LC, Silva I, Henriques SS, Silva RRD, Sousa P (2008a) Assessing spider species richness and composition in Mediterranean cork oak forests. Acta Oecologica 33:114-127.

Cardoso P, Henriques SS, Gaspar C, Crespo LC, Carvalho R, Schmidt JB, Sousa P, Szüts T (2007a) Species richness and composition assessment of spiders in a Mediterranean scrubland. Journal of Insect Conservation 13:45-55.

Cardoso P, Morano E (2010) The Iberian spider checklist (Araneae). Zootaxa:1-52.

Cardoso P, Pekár S, Jocqué R, Coddington JA Guild composition and functional diversity of spiders at a global scale. (submitted).

Cardoso P, Scharff N, Gaspar C, Henriques SS, Carvalho R, Castro PH, Schmidt JB, Silva I, Szüts T, Castro AD, Crespo LC (2008b) Rapid biodiversity assessment of spiders (Araneae) using semi-quantitative sampling: a case study in a Mediterranean forest. Insect Conservation and Diversity 1:71-84.

Cardoso P, Silva I, Oliveira NGD, Serrano ARM (2004) Indicator taxa of spider (Araneae) diversity and their efficiency in conservation. Biological Conservation 120:517-524.

Cardoso P, Silva I, Oliveira NGD, Serrano ARM (2007b) Seasonality of spiders (Araneae) in Mediterranean ecosystems and its implications in the optimum sampling period. Ecological Entomology 32:516-526.

Cerveira AM, Jackson RR (2005) Specialised predation by Palpimanus sp. (Araneae: Palpimanidae) on jumping spiders (Araneae: Salticidae). Journal of East African Natural History 94:303-317.

Chatzaki M, Trichas A, Markakis G, Mylonas M (1998) Seasonal activity of the ground spider fauna in a Mediterranean ecosystem (Mt Youchtas, Crete, Greece). In: P. A. Selden (eds) Proceedings of the 17th European Colloquium of Arachnology, Edinburgh 1997. Edinburgh, pp 235-243.

Conti ME, Cecchetti G (2001) Biological monitoring: lichens as bioindicators of air pollution assessment - a review. Environmental Pollution 114:471-492.

Correia O, Branquinho C, Antunes C, Gato JTVD (2009) Relatório final referente ao Protocolo entre a CMA e o Centro de Biologia Ambiental (FCUL). Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal.

Dean DA, Sterling WL (1985) Size and phenology of ballooning spiders at two locations in eastern Texas. Journal of Arachnology 13:111–120.

Edwards CA, Subler S, Chen SK, Bogomolov DM (1996) Essential criteria for selecting bioindicator species, processes or systems of the impact of chemicals in the soil. In: Straalen NMV, Krivolutsky DA (eds) Bioindicator Systems for Soil Pollution. Springer, Moscow, Russia, pp 67-84.

Eeva T, Sorvari J, Koivunen V (2004) Effects of heavy metal pollution on red wood ant (Formica s. str.) populations. Environmental Pollution 132:533-9.

Eraly D, Hendrickx F, Bervoets L, Lens L (2010) Experimental exposure to cadmium affects metallothionein-like protein levels but not survival and growth in wolf spiders from polluted and reference populations. Environmental Pollution 158:2124-2131.

Eraly D, Hendrickx F, Lens L (2009) Condition-dependent mate choice and its implications for population differentiation in the wolf spider Pirata piraticus. Behavioral Ecology 20:856-863.

Filzek PDB, Spurgeon DJ, Broll G, Svendsen C, Hankard PK, Kammenga JE, Donker MH, Weeks JM (2004) Pedological characterisation of sites along a transect from a primary cadmium/lead/zinc smelting works. Ecotoxicology 13:725-737.

Foelix RF (1996) Biology of Spiders. Oxford University Press, USA.

Franzle S, Markert B (2008) The biological system of elements: trace element concentration and abundance in plants give hints on biochemical reasons of sequestration and essentiality. In: Prasad MNV (ed) Trace Elements as Contaminants and Nutrients: Consequences in Ecosystems and Human Health. Wiley, pp 29-49.

Greenstone MH, Morgan CE, Hultsch A-L, Farrow RA, Dowse JE (1987) Ballooning spiders in Missouri, USA, and New South Wales, Australia: family and mass distributions. Journal of Arachnology 15:163-170.

Heikens A, Peijnenburg W, Hendriks AJ (2001) Bioaccumulation of heavy metals in terrestrial invertebrates. Environmental Pollution 113:385-393.

Hendrickx F, Maelfait J-P, Bogaert N, Tojal C, Laing GD, Tack FMG, Verloo MG (2004) The importance of biological factors affecting trace metal concentration as revealed from accumulation patterns in co-occurring terrestrial invertebrates. Environmental Pollution 127:335-341.

Hendrickx F, Maelfait J-P, Langenbick F (2003a) Absence of cadmium excretion and high assimilation result in cadmium biomagnification in a wolf spider. Ecotoxicology and Environmental Safety 55:287-292.

Hendrickx F, Maelfait J-P, Lens L (2008) Effect of metal stress on life history divergence and quantitative genetic architecture in a wolf spider. Journal of evolutionary biology 21:183-93.

Hendrickx F, Maelfait J-P, Mayer AD, Tack FMG, Verloo MG (2003b) Storage mediums affect metal concentration in woodlice (Isopoda). Environmental Pollution 121:87-93.

Hendrickx F, Maelfait J-P, Speelmans M, Straalen NMV (2003c) Adaptive reproductive variation along a pollution gradient in a wolf spider. Oecologia 134:189-94.

Hopkin SP, Martin MH (1985) Assimilation of zinc, cadmium, lead, copper, and iron by the spider Dysdera crocata, a predator of woodlice. Bulletin of Environmental Contamination and Toxicology 34:183-187.

Hunter BA, Johnson MS, Thompson DJ (1987a) Ecotoxicology of copper and cadmium in a contaminated grassland ecosystem: I. Soil and vegetation contamination. Journal of Applied Ecology 24:573-586.

Hunter BA, Johnson MS, Thompson DJ (1987b) Ecotoxicology of copper and cadmium in a contaminated grassland ecosystem: II. Invertebrates. Journal of Applied Ecology 24:587-599.

Hunter BA, Johnson MS, Thompson DJ (1987c) Ecotoxicology of copper and cadmium in a contaminated grassland ecosystem: III. Small mammals. The Journal of Applied Ecology 24:601-614.

Janssen MP, Hogervorst R (1993) Metal accumulation in soil arthropods in relation to micro-nutrients. Environmental pollution (Barking, Essex : 1987) 79:181-9.

Jiménez-Valverde A, Lobo JM (2006) Establishing reliable spider (Araneae, Araneidae and Thomisidae) assemblage sampling protocols: estimation of species richness, seasonal coverage and contribution of juvenile data to species richness and composition. Acta Oecologica 30:21-32.

Jung M-P, Kim S-T, Kim H, Lee J-ho (2008) Species diversity and community structure of ground-dwelling spiders in heavy metal-polluted habitats. Water, Air, and Soil Pollution 195:15-22.

Kabata-Pendias A (2004) Soil-plant transfer of trace elements — an environmental issue. Geoderma 122:143-149.

Kabata-Pendias A, Mukherjee AB (2007) Trace elements from Soil to Human. Springer, Berlin.

Kuperman RG, Carreiro MM (1997) Soil heavy metal concentrations, microbial biomass and enzyme activities in a contaminated grassland ecosystem. Soil Biology and Biochemistry 29:179-190.

Laing GD, Bogaert N, Tack FMG, Verloo MG, Hendrickx F (2002) Heavy metal contents (Cd, Cu, Zn) in spiders (Pirata piraticus) living in intertidal sediments of the river Scheldt estuary (Belgium) as affected by substrate characteristics. The Science of The Total Environment 289:71-81.

Lang A (2000) The pitfalls of pitfalls: a comparison of pitfall trap catches and absolute density estimates of epigeal invertebrate predators in arable land. Anzeiger für Schädlingskunde 73:99-106.

Lee MY, Huebers H, Martin AW, Finch CA (1978) Iron metabolism in a spider, Dugesiella hentzi. Journal of Comparative Physiology B: Biochemical, Systemic and Environmental Physiology 127:349-354.

Love A, Babu CR (2006) Trophic transfer of trace elements and associate human health effects. In: Prasad MNV, Sajwan KS, Naidu R (eds) Trace Elements in the Environment (Biogeochemistry, biotechnology and bioremediation). Taylor & Francis Group, Boca Raton, Florida, pp 659-688.

Maelfait J-P (1996) Soil spiders and bioindication. In: Straalen NMV, Krivolutsky DA (eds) Bioindicator Systems for Soil Pollution. Springer, Moscow, Russia, pp 165-178.

Marc P, Canard A, Ysnel F (1999) Spiders (Araneae) useful for pest limitation and bioindication. Agriculture, Ecosystems & Environment 74:229-273.

Marczyk G, Migula P, Trzcionka E (1993) Physiological responses of spiders to environmental pollution in the Silesian Region (Southern Poland). Science of The Total Environment 134:1315-1322.

Marmiroli N, Maestri E (2008) Health implications of trace elements in the environment and the food chain. In: Prasad MNV (ed) Trace Elements as Contaminants and Nutrients: Consequences in Ecosystems and Human Health. Wiley,.

Melbourne BA (1999) Bias in the effect of habitat structure on pitfall traps: An experimental evaluation. Austral Ecology 24:228-239.

Ministério do Ambiente (1996) Portaria 176/96. 10-12.

Nahmani J, Lavelle P (2002) Effects of heavy metal pollution on soil macrofauna in a grassland of Northern France. European Journal of Soil Biology 38:297-300.

Norris KC (1999) Quantifying change through time in spider assemblages: sampling methods, indices and sources of error. Journal of Insect Conservation 3:309–325.

Pearce S, Zalucki MP, Hassan E (2005) Spider ballooning in soybean and non-crop areas of southeast Queensland. Agriculture, Ecosystems and Environment 105:273-281.

Pekár S (2004) Predatory behavior of two European ant-eating spiders (Araneae, Zodariidae). Journal of Arachnology 32:31-41.

Pekár S, Hrušková M, Lubin Y (2004) Can solitary spiders (Araneae) cooperate in prey capture? Journal of Animal Ecology 74:63-70.

Pinho P, Augusto S, Martins-Loução MA, Pereira MJ, Soares A, Máguas C, Branquinho C (2008) Causes of change in nitrophytic and oligotrophic lichen species in a Mediterranean climate: impact of land cover and atmospheric pollutants. Environmental Pollution 154:380-9.

Rabitsch WGB (1995) Metal accumulation in arthropods near a lead/zinc smelter in Arnoldstein, Austria: III. Arachnida. Environmental Pollution 90:221-237.

Rypstra AL, Carter PE, Balfour RA, Marshall SD (1999) Architectural features of agricultural habitats and their impact on the spider inhabitants. Journal of Arachnology 27:371-377.

Schipper AM, Wijnhoven S, Leuven RSEW, Ragas AMJ, Hendriks AJ (2008) Spatial distribution and internal metal concentrations of terrestrial arthropods in a moderately contaminated lowland floodplain along the Rhine River. Environmental Pollution 151:17-26.

Simberloff D, Dayan T (1991) The Guild Concept and the Structure of Ecological Communities. Annual Review of Ecology and Systematics 22:115-143.

Spurgeon DJ, Sandifer RD, Hopkin SP (1996) The use of macroinvertebrates for population and community monitoring of metal contamination. In: Straalen NMV, Krivolutsky DA (eds) Bioindicator Systems for Soil Pollution. Springer, pp 95-110.

Spurgeon DJ, Jones OAH, Dorne J-LCM, Svendsen C, Swain S, Stürzenbaum SR (2010) Systems toxicology approaches for understanding the joint effects of environmental chemical mixtures. The Science of the Total Environment 408:3725-3734.

Straalen NMV, Butovsky RO, Pokarzhevskii AD, Zaitsev AS, Verhoef SC (2001) Metal concentrations in soil and invertebrates in the vicinity of a metallurgical factory near Tula (Russia). Pedobiologia 45:451-466.

Tavares CDA (2007) Spider (Arachnida: Araneae) on pear orchards in the "Oeste" region of Portugal. MSc Thesis:1-40.

Topping CJ, Sunderland KD (1992) Limitations to the use of pitfall traps in ecological studies exemplified by a study of spiders in a field of winter wheat. Journal of applied ecology 29:485–491.

Tyler G, Pahlsson AMB, Bengtsson G, Baath E, Tranvik L (1989) Heavy-metal ecology of terrestrial plants, microorganisms and invertebrates. Water, Air, and Soil Pollution 47:189-215.

Uetz GW (1977) Coexistence in a guild of wandering spiders. The Journal of Animal Ecology 46:531-541.

Uetz GW, Halaj J, Cady AB (1999) Guild structure of spiders in major crops. Journal of Arachnology 27:270-280.

Wilczek G, Babczyńska A, Augustyniak M, Migula P (2004) Relations between metals (Zn, Pb, Cd and Cu) and glutathione-dependent detoxifying enzymes in spiders from a heavy metal pollution gradient. Environmental Pollution 132:453-61.

Wise DH (1993) Spiders in Ecological Webs (HJB Birks and JA Wiens, Eds.). Cambridge University Press, Cambridge, United Kingdom.

Abbreviations

Cu – Copper

Fe – Iron

GnaphoCu – Gnaphosidae Copper Contents

GnaphoFe – Gnaphosidae Iron Contents

GnaphoZn – Gnaphosidae Zinc Contents

HSCS – Habitat Structural Complexity Score

LBioCu – Lichens Bioavailable Copper

LDV – Lichen Diversity Value

LPartCu – Lichens Particulate Copper

SBioCu – Soil Bioavailable Copper

SBioFe – Soil Bioavailable Iron

SBioZn – Soil Bioavailable Zinc

Zn – Zinc

Annex I

Table 1. Guild classification of all identified families in both Spring and Autumn samplings according to Cardoso et al. (subm.).

	Guilds	Orb Web weavers	Sheet Web weavers	Space Web weavers	Sensing Web weavers	Specialists	Ground Hunters	Ambush Hunters	Other Hunters
Families	***Sub-families***								
Agelenidae			X						
Araneidae		X							
Atypidae					X				
Clubionidae									X
Corinnidae							X		
Ctenizidae					X				
Dictynidae	Dictyninae			X					
	Cicurininae						X		
	Tricholathysinae						X		
Dysderidae						X			
Filistatidae					X				
Gnaphosidae							X		
Linyphiidae	Linyphiinae		X						
	Other subfamilies								X
Liocranidae							X		
Lycosidae							X		
Miturgidae									X
Nemesiidae					X				
Oecobiidae					X				
Oonopidae							X		
Oxyopidae									X
Palpimanidae						X			
Philodromidae									X
Pholcidae				X					
Pisauridae			X						
Prodidomidae							X		
Salticidae									X
Scytodidae									X
Sicariidae								X	
Sparassidae									X
Theridiidae				X					
Thomisidae								X	
Zodariidae						X			
Zoridae							X		

Table 2. Dry weight of Gnaphosidae juvenile, adult female and male specimens used in the pooled samples for heavy metal analysis.

Site	Sex/Maturity	Number of specimens	Dry weight (g)	Dry weight contribution to pooled sample (%)	Concentration of Cu (µg/g DW)	Concentration of Fe (µg/g DW)	Concentration of Zn (µg/g DW)
B-01	Juveniles	2	0.0019	28			
B-01	Adult females	1	0.0031	46			
B-01	Adults males	1	0.0017	25			
B-01	***Pooled sample***	**4**	**0.0067**		**409.59**	**1053.36**	**528.13**
B-02	Juveniles	4	0.0123	40			
B-02	Adult females	2	0.0186	60			
B-02	***Pooled sample***	**6**	**0.0309**		**317.33**	**388.67**	**674.50**
B-03	Juveniles	2	0.0012	100			
B-03	***Pooled sample***	**2**	**0.0012**		**176.88**	**801.04**	**371.88**
B-04	Juveniles	5	0.0068	44			
B-04	Adult females	2	0.0059	39			
B-04	Adult males	1	0.0026	17			
B-04	***Pooled sample***	**8**	**0.0153**		**431.32**	**847.94**	**782.25**
A-05	Juveniles	2	0.0115	50			
A-05	Adult females	3	0.0113	50			
A-05	***Pooled sample***	**5**	**0.0228**		**304.67**	**357.46**	**440.96**
A-06	Juveniles	7	0.0436	73			
A-06	Adult females	2	0.0160	27			
A-06	***Pooled sample***	**9**	**0.0596**		**81.17**	**194.19**	**357.75**
A-07	Juveniles	1	0.0024	5			
A-07	Adult females	6	0.0276	55			
A-07	Adult males	5	0.0200	40			
A-07	***Pooled sample***	**12**	**0.0500**		**224.55**	**289.58**	**705.38**
B-08	Juveniles	1	0.0078	39			
B-08	Adult females	2	0.0081	41			
B-08	Adult males	2	0.0040	20			
B-08	***Pooled sample***	**5**	**0.0199**		**128.18**	**130.63**	**304.15**
B-09	Adult females	4	0.0241	91			
B-09	Adult males	1	0.0023	9			
B-09	***Pooled sample***	**5**	**0.0264**		**251.19**	**345.23**	**871.59**
A-11	Adult females	1	0.0039	50			
A-11	Adult males	1	0.0039	50			
A-11	***Pooled sample***	**2**	**0.0078**		**175.29**	**179.81**	**452.50**
A-12	Juveniles	1	0.0002	4			
A-12	Adult females	1	0.0054	96			
A-12	***Pooled sample***	**2**	**0.0056**		**169.96**	**238.66**	**436.88**
A-13	Juveniles	4	0.0223	84			
A-13	Adult females	1	0.0042	16			
A-13	***Pooled sample***	**5**	**0.0265**		**110.21**	**116.07**	**378.25**
B-14	Juveniles	2	0.0245	72			
B-14	Adult females	2	0.0095	28			
B-14	***Pooled sample***	**4**	**0.0340**		**104.69**	**261.07**	**344.32**
B-15	Juveniles	2	0.0015	24			
B-15	Adult females	1	0.0047	76			
B-15	***Pooled sample***	**3**	**0.0062**		**204.31**	**271.69**	**474.44**
A-16	Juveniles	2	0.0042	27			
A-16	Adult females	2	0.0085	55			
A-16	Adult males	1	0.0028	18			
A-16	***Pooled sample***	**5**	**0.0155**		**273.44**	**584.03**	**560.42**
A-19	Juveniles	1	0.0032	15			
A-19	Adult females	2	0.0076	36			
A-19	Adult males	4	0.0105	49			
A-19	***Pooled sample***	**7**	**0.0213**		**289.93**	**361.60**	**485.35**
	Juveniles	**36**	**0.1434**	**41**			
	Adult females	**32**	**0.1585**	**45**			
	Adult males	**16**	**0.0478**	**14**			
All		**84**	**0.3497**	***Average:***	228.29	401.31	510.55
				Standard-Error:	104.74	276.48	166.87

Table 2. Qualitative habitat characterization of the sampling sites for the time periods during which the data presented in this study was collected. Sites are ordered by season and then by distance to the mine centre. Scores per habitat structure category were given by comparison to two sites among all the sampled ones that could be considered as having the lowest and the highest score within that category: 0 – Non-existent; 1 – Scarse; 2 – Moderate; 3 – Abundant/Dense. The Habitat Sctructural Compelxity Score (HSCS) is the sum of all the scores of the five described structural aspects.

Sampling Sites	Season	Sampling (every 13 days)	Directional Axis (N, S, E, W)	Elevation (meters)	Distance to Mine Centre (meters)	Land Use (categories)	Trampling (categories)	Tillage (Y/N)	Soil Texture (categories)	Soil Humidity (categories)	Ground shelters (score)	Litter layer (score)	Herba-ceous layer (score)	Shrub layer (score)	Arboreal layer (score)	Habitat Structural Complexity Score (total score)
B-01	Spring	2nd	C	215	219.42	Industrial	Industrial	N	Compact and rocky	Very Dry	2	1	0	1	1	**5**
S-10	Spring	2nd	W	201	344.51	Unmanaged land	Human	N	Loose soil	Moist	3	3	1	2	2	**11**
S-11	Spring	2nd	W	210	511.82	Unmanaged land	Human	Y	Bulky, ploughed soil	Very Dry	3	1	3	1	1	**9**
B-09	Spring	2nd	W	219	690.20	Cattle Grazing	Ovine cattle	Y	Resting ploughed soil	Very Dry	2	1	1	1	1	**6**
B-14	Spring	2nd	W	224	1144.69	Rock-Rose land	Negligible	N	Compact and rocky	Very Dry	2	0	1	3	1	**7**
B-01	Autumn	2nd	C	215	219.42	Industrial	Industrial	N	Compact and rocky	Dry	2	1	0	1	1	**5**
B-02	Autumn	2nd	C	220	233.08	Industrial	Industrial	N	Compact and rocky	Dry	2	1	0	1	1	**5**
B-04	Autumn	2nd	E	199	418.41	Industrial	Human	N	Muddy, powdery soil	Moist	0	0	1	0	1	**2**
B-03	Autumn	2nd	N	224	463.63	Industrial	Industrial	N	Loose soil	Moist	3	3	1	3	1	**11**
A-16	Autumn	2nd	E	199	567.57	Cattle Grazing	Ovine cattle	N	Powdery soil	Moist	0	0	0	1	0	**1**
A-06	Autumn	2nd	S	199	686.39	Cattle Grazing	Ovine cattle	Y	Loose rich soil	Humid	3	1	3	1	0	**8**
B-09	Autumn	2nd	W	220	690.20	Cattle Grazing	Ovine cattle	Y	Resting ploughed soil	Moist	2	1	1	1	1	**6**
A-11	Autumn	2nd	W	206	702.50	Cattle Grazing	Ovine cattle	Y	Resting ploughed soil	Moist	3	2	2	2	2	**11**
B-08	Autumn	2nd	NE	221	880.17	Unmanaged land	Human	N	Compact and rocky	Dry	3	1	2	1	1	**8**
A-05	Autumn	2nd	E	199	1028.19	Cattle Grazing	Cattle + Vehicles	N	Powdery soil	Moist	1	2	1	0	3	**7**
A-13	Autumn	2nd	N	232	1066.85	Cattle Grazing	Ovine cattle	Y	Bulky, ploughed soil	Dry	1	0	1	1	1	**4**
A-19	Autumn	2nd	NW	224	1073.67	Resting land	Human	Y	Bulky, ploughed soil	Dry	3	2	2	2	2	**11**
B-15	Autumn	2nd	S	199	1115.28	Cattle Grazing	Bovine cattle	Y	Powdery soil	Moist	1	0	2	1	0	**4**
B-14	Autumn	2nd	W	224	1144.69	Rock-Rose land	Negligible	N	Compact and rocky	Dry	2	0	1	3	1	**7**
A-07	Autumn	2nd	S	215	1604.97	Agricultural	Agricultural + Cattle	Y	Bulky, ploughed soil	Dry	2	0	1	0	1	**4**
A-12	Autumn	2nd	E	200	1966.34	Cattle Grazing	Bovine cattle	Y	Bulky, ploughed soil	Dry	2	1	2	0	1	**6**

Annex II

Table 1. Spearman correlations to ascertain whether characteristics of the samples for spider heavy metal content analysis significantly influenced results. Statistically significant results at $p<0.05$ are in blue.

Correlations	Spearman R	p-value
Number of specimens & Cu Contents	0.244	0.362
Number of specimens & Fe Contents	0.111	0.683
Number of specimens & Zn Contents	0.364	0.166
Pooled Sample DW & Cu Contents	-0.221	0.412
Pooled Sample DW & Fe Contents	-0.297	0.264
Pooled Sample DW & Zn Contents	0.012	0.966
DW Contribution of Juveniles (%) & Cu Contents	-0.209	0.437
DW Contribution of Juveniles (%) & Fe Contents	0.062	0.820
DW Contribution of Juveniles (%) & Zn Contents	-0.515	0.041
DW Contribution of Females (%) & Cu Contents	0.300	0.259
DW Contribution of Females (%) & Fe Contents	0.032	0.905
DW Contribution of Females (%) & Zn Contents	0.541	0.030
DW Contribution of Males (%) & Cu Contents	0.309	0.243
DW Contribution of Males (%) & Fe Contents	0.127	0.639
DW Contribution of Males (%) & Zn Contents	0.375	0.152
DW Contribution of Adults (%) & Cu Contents	0.209	0.437
DW Contribution of Adults (%) & Fe Contents	-0.062	0.820
DW Contribution of Adults (%) & Zn Contents	0.515	0.041

Table 2. Model fitting r values. Significant values on a 95% confidence interval (N=16; r = 0.426; R-square = 0.181476) are in black; non-significant values are in light blue. Models with an explainability of over 40% (R-square > 0.40) are in bold.

r	Variable:	Distance		LBioCu		LPartCu		SBioCu		SBioFe		SBioZn		GnaphoCu		GnaphoFe		GnaphoZn		LDV	
N	Sign / Type of model:	Sign	Log.	Sign	Linear	Sign	Linear	Sign	Log.	Sign	Log.	Sign	Log.	Sign	Linear	Sign	Linear	Sign	Linear	Sign	Linear
34	Sheet Web: AGELENI. Juveniles	+	0.313	-	0.276	-	0.257	-	0.204	-	0.340	-	0.267	-	0.041	-	0.288	+	0.037	+	0.294
60	Sheet Web: AGELENIDAE	+	0.096	-	0.405	-	0.400	-	0.141	-	0.252	-	0.220	+	0.145	-	0.032	+	0.075	+	0.253
34	**Sheet Web: Juveniles**	+	0.313	-	0.276	-	0.257	-	0.204	-	0.340	-	0.267	-	0.041	-	0.288	+	0.037	+	0.294
60	**Sheet Web: All**	+	0.096	-	0.405	-	0.400	-	0.141	-	0.252	-	0.220	+	0.145	-	0.032	+	0.075	+	0.253
101	Sensing Web: NEMESII. Males	+	0.217	+	0.042	+	0.119	+	0.015	-	0.180	-	0.369	-	0.066	-	0.127	-	0.082	-	0.176
104	Sensing Web: NEMESII. Adults	+	0.216	+	0.044	+	0.121	+	0.012	-	0.181	-	0.373	-	0.066	-	0.128	-	0.081	-	0.174
109	Sensing Web: NEMESIIDAE	+	0.227	+	0.028	+	0.105	+	0.003	-	0.192	-	0.384	-	0.071	-	0.136	-	0.089	-	0.167
32	**Sensing Web: Juveniles**	+	0.266	-	0.452	-	0.569	-	0.485	-	0.191	-	0.386	-	0.263	-	0.298	-	0.200	+	0.524
112	**Sensing Web: Males**	+	0.234	+	0.025	+	0.103	+	0.011	-	0.172	-	0.365	-	0.098	-	0.117	-	0.116	-	0.151
115	**Sensing Web: Adults**	+	0.231	+	0.028	+	0.106	+	0.009	-	0.174	-	0.369	-	0.097	-	0.118	-	0.113	-	0.151
147	**Sensing Web: All**	+	0.307	-	0.097	-	0.051	-	0.126	-	0.228	-	0.479	-	0.171	-	0.202	-	0.170	-	0.007
147	Specialists: ZODARII. Juveniles	+	**0.698**	-	**0.686**	-	0.532	-	0.547	-	0.411	-	0.514	-	0.259	-	0.578	-	0.175	+	0.285
47	Specialists: ZODARII. Females	+	0.187	-	0.397	-	**0.604**	-	0.283	+	0.184	-	0.173	-	0.471	-	0.519	-	0.328	+	0.566
197	Specialists: ZODARII. Males	+	0.125	-	0.367	-	**0.673**	-	0.112	+	0.258	-	0.017	-	0.374	-	0.466	-	0.318	+	0.478
244	Specialists: ZODARII. Adults	+	0.139	-	0.377	-	**0.665**	-	0.149	+	0.245	-	0.049	-	0.397	-	0.482	-	0.324	+	0.501
391	Specialists: ZODARIIDAE	+	0.355	-	0.540	-	**0.714**	-	0.310	+	0.050	-	0.220	-	0.406	-	0.585	-	0.318	+	0.497
157	**Specialists: Juveniles**	+	**0.682**	-	**0.712**	-	0.569	-	0.566	-	0.414	-	0.526	-	0.250	-	0.561	-	0.179	+	0.311
48	**Specialists: Females**	+	0.199	-	0.405	-	**0.613**	-	0.279	+	0.181	-	0.165	-	0.465	-	0.530	-	0.340	+	0.558
199	**Specialists: Males**	+	0.131	-	0.379	-	**0.678**	-	0.121	+	0.247	-	0.026	-	0.376	-	0.468	-	0.323	+	0.488
247	**Specialists: Adults**	+	0.146	-	0.388	-	**0.672**	-	0.154	+	0.236	-	0.055	-	0.398	-	0.485	-	0.330	+	0.507
404	**Specialists: All**	+	0.363	-	0.559	-	**0.724**	-	0.326	+	0.029	-	0.235	-	0.397	-	0.578	-	0.318	+	0.503
230	Ground Hunters: GNAPHOSI. Juveniles	+	0.288	+	0.236	+	0.487	-	0.333	-	0.303	-	0.357	-	0.309	-	0.368	-	0.141	+	0.009
112	Ground Hunters: GNAPHOSI. Females	+	**0.614**	-	**0.619**	-	0.089	-	**0.610**	-	0.565	-	0.592	-	0.252	-	0.423	-	0.120	+	0.408
244	Ground Hunters: GNAPHOSI. Males	+	0.521	-	**0.695**	-	0.583	-	0.456	-	0.217	-	0.283	-	0.261	-	0.468	-	0.208	+	0.471
356	Ground Hunters: GNAPHOSI. Adults	+	**0.678**	-	**0.816**	-	0.491	-	**0.627**	-	0.421	-	0.485	-	0.315	-	0.552	-	0.107	+	0.548
586	Ground Hunters: GNAPHOSIDAE	+	**0.749**	-	0.460	-	0.016	-	**0.743**	-	0.559	-	**0.649**	-	0.481	-	**0.711**	-	0.190	+	0.436
42	Ground Hunters: LYCOSI. Juveniles	-	0.216	+	0.385	+	0.472	+	0.050	-	0.375	-	0.029	+	0.186	+	0.248	-	0.175	-	0.305
45	Ground Hunters: LYCOSIDAE	-	0.238	+	0.391	+	0.464	+	0.087	-	0.363	-	0.006	+	0.229	+	0.287	-	0.164	-	0.346

Table 2. (continued)

r	Variable:	Distance		LBioCu		LPartCu		SBioCu		SBioFe		SBioZn		GnaphoCu		GnaphoFe		GnaphoZn		LDV	
N	Sign / Type of model:	Sign	Log.	Sign	Linear	Sign	Linear	Sign	Log.	Sign	Log.	Sign	Log.	Sign	Linear	Sign	Linear	Sign	Linear	Sign	Linear
280	Ground Hunters: Juveniles	+	0.221	+	0.247	+	0.474	-	0.326	-	0.353	-	0.345	-	0.260	-	0.289	-	0.179	+	0.007
115	Ground Hunters: Females	+	**0.610**	-	**0.634**	-	0.091	-	**0.601**	-	0.553	-	0.585	-	0.248	-	0.436	+	0.117	+	0.411
261	Ground Hunters: Males	+	0.509	-	**0.717**	-	0.567	-	0.472	-	0.236	-	0.300	-	0.302	-	0.451	-	0.250	+	0.513
376	Ground Hunters: Adults	+	**0.657**	-	**0.827**	-	0.473	-	**0.625**	-	0.423	-	0.486	-	0.340	-	0.537	-	0.139	+	0.573
656	Ground Hunters: All	+	**0.677**	-	0.403	+	0.054	-	**0.743**	-	**0.616**	-	**0.656**	-	0.475	-	**0.646**	-	0.256	+	0.432
45	Ambush Hunters: THOMISI. Juveniles	+	0.310	+	0.161	+	0.319	-	0.075	-	0.242	-	0.157	-	0.049	-	0.195	-	0.172	-	0.329
64	Ambush Hunters: THOMISI. Males	+	0.347	-	0.292	-	0.219	-	0.233	-	0.113	+	0.020	+	0.151	-	0.038	-	0.011	-	0.371
79	Ambush Hunters: THOMISI. Adults	+	0.392	-	0.334	-	0.212	-	0.023	-	0.206	-	0.095	+	0.148	-	0.105	+	0.022	-	0.360
124	Ambush Hunters: THOMISIDAE	+	0.453	-	0.176	-	0.005	-	0.100	-	0.277	-	0.150	+	0.089	-	0.177	-	0.069	-	0.438
50	Ambush Hunters: Juveniles	+	0.405	+	0.040	+	0.213	-	0.141	-	0.287	-	0.206	-	0.141	-	0.273	-	0.267	-	0.260
67	Ambush Hunters: Males	+	0.362	-	0.331	-	0.257	-	0.039	-	0.129	-	0.009	+	0.136	-	0.026	-	0.020	-	0.336
82	Ambush Hunters: Adults	+	0.404	-	0.367	-	0.246	-	0.097	-	0.220	-	0.107	+	0.134	-	0.095	-	0.006	-	0.326
132	Ambush Hunters: All	+	0.497	-	0.253	-	0.078	-	0.141	-	0.303	-	0.180	+	0.030	-	0.203	-	0.135	-	0.368
30	Other Hunters: LINYPHII. Females	-	0.090	+	0.350	+	0.279	+	0.101	+	0.132	+	0.185	+	0.194	+	0.065	+	0.110	-	0.216
131	Other Hunters: LINYPHII. Males	-	0.100	+	0.487	+	0.587	-	0.010	+	0.028	+	0.165	-	0.159	-	0.140	-	0.015	-	0.045
161	Other Hunters: LINYPHII. Adults	-	0.104	+	0.492	+	0.565	+	0.011	+	0.049	+	0.179	-	0.102	-	0.110	+	0.008	-	0.080
174	Other Hunters: LINYPHIIDAE	-	0.054	+	0.444	+	0.487	-	0.060	+	0.055	+	0.138	-	0.144	-	0.154	-	0.006	-	0.013
131	Other Hunters: SALTICI. Males	-	0.199	-	0.319	-	0.288	+	0.020	-	0.152	-	0.038	+	0.080	+	0.129	-	0.192	+	0.263
132	Other Hunters: SALTICI. Adults	-	0.210	-	0.310	-	0.282	+	0.025	-	0.157	-	0.034	+	0.091	+	0.143	-	0.190	+	0.254
135	Other Hunters: SALTICIDAE	-	0.201	-	0.315	-	0.288	+	0.018	-	0.158	-	0.037	+	0.082	+	0.145	-	0.198	+	0.263
63	Other Hunters: Juveniles	+	0.453	-	0.543	-	0.498	-	0.502	-	0.142	-	0.456	-	0.459	-	0.362	-	0.373	+	0.432
31	Other Hunters: Females	-	0.149	+	0.388	+	0.301	+	0.127	+	0.110	+	0.207	+	0.248	+	0.135	+	0.115	-	0.254
264	Other Hunters: Males	-	0.265	+	0.167	+	0.265	+	0.020	-	0.088	+	0.128	-	0.078	+	0.002	-	0.186	+	0.184
295	Other Hunters: Adults	-	0.277	+	0.227	-	0.304	+	0.042	-	0.064	+	0.158	-	0.029	+	0.026	-	0.155	+	0.129
358	Other Hunters: All	-	0.187	+	0.113	+	0.205	-	0.072	-	0.100	+	0.061	-	0.137	-	0.057	-	0.250	+	0.236
617	All Juveniles	+	**0.623**	-	0.304	-	0.083	-	**0.638**	-	0.529	-	**0.627**	-	0.403	-	0.589	-	0.308	+	0.271
213	All Females	+	**0.633**	-	**0.665**	-	0.318	-	**0.663**	-	0.424	-	**0.638**	-	0.375	-	**0.672**	-	0.059	+	0.538
930	All Males	+	0.370	-	0.588	-	**0.620**	-	0.291	-	0.110	-	0.303	-	0.467	-	**0.602**	-	0.533	+	0.546
1143	All Adults	+	0.466	-	**0.653**	-	0.590	-	0.409	-	0.200	-	0.412	-	0.480	-	**0.666**	-	0.452	+	0.586
1760	All	+	**0.677**	-	**0.618**	-	0.447	-	**0.647**	-	0.443	-	**0.643**	-	0.559	-	**0.793**	-	0.485	+	0.553